Biological Effects and Implications of Micro- and Nanoplastics in the Aquatic Environment

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Preface

The work presented in this PhD thesis was carried out at the Department of Environmental Engineering at the Technical University of Denmark (DTU) from December 2015 to December 2018 under the supervision of Professor Anders Baun and Senior Researcher Nanna B. Hartmann.

The thesis is organized in two parts: the first part puts into context the findings of the PhD in an introductive review; the second part consists of the papers listed below. These will be referred to in the text by their paper number written with the Roman numerals **I-VIII**.

I **Rist, S.**, Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in *Daphnia magna* – Quantification of body burdens and assessment of feeding rates and reproduction. *Environmental Pollution*. 228, 398-407. doi:10.1016/j.envpol.2017.05.048

II **Rist, S.**, Carney Almroth, B., Hartmann, N.B., Karlsson, T.M., 2018. A critical perspective on early communications concerning human health aspects of microplastics. *Science of the Total Environment*. 626, 720–726. doi:10.1016/j.scitotenv.2018.01.092

III **Rist, S.**, Hartmann, N.B., 2018. Aquatic Ecotoxicology of Microplastics and Nanoplastics: Lessons Learned from Engineered Nanomaterials. In: Wagner, M., Lambert, S. (Eds.), Freshwater Microplastics. The Handbook of Environmental Chemistry, vol 58. *Springer.* Cham, pp. 25-49. doi:10.1007/978-3-319-61615-5_2

IV **Rist, S.**, Steensgaard, I.M., Guven, O., Gissel Nielsen, T., Jensen, L.H., Møller, L.F., Hartmann, N.B. 2018. The Fate of Microplastics During Uptake and Depuration Phases in a Blue Mussel Exposure System. *Environmental Toxicology and Chemistry*. Published online 1 Oct 2018. doi:10.1002/etc.4285

V **Rist, S.**, Baun, A., Almeda, R., Hartmann, N.B., 2018. Ingestion and effects of micro- and nanoplastics in blue mussel (*Mytilus edulis*) larvae. *Submitted.*

VI Schür, C., **Rist, S.**, Baun, A., Hartmann, N.B., Wagner, M., 2018. Tissue translocation of fluorescent polystyrene microplastics in *Daphnia magna* – An artefact of leaching dye? *Manuscript*. (joint first authors)

VII Hartmann, N.B., **Rist, S.**, Bodin, J., Jensen, L.H., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as Vectors for Environmental Contaminants: Exploring Sorption, Desorption, and Transfer to Biota. *Integrated Environmental Assessment and Management*. 13, 488–493. doi:10.1002/ieam.1904

VIII Hartmann, N.B., Hüffer, T., Thompson, R., Hassellöv, M., Verschoor, A., Daugaard, A.E., **Rist, S.**, Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Heß, M., Ivleva, N.P., Lusher, A.L., Wagner, M., 2018. Are we speaking the same language? Towards a definition and categorization framework for environmental plastic debris. *Submitted.*

In this online version of the thesis, paper **I-VIII** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following publications, not included in this thesis, were also concluded during this PhD study:

Rist, S., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M. Lenz, M. 2016. Suspended micro-sized PVC particles impair the performance and decrease survival on the Asian green mussel *Perna viridis*. *Marine Pollution Bulletin*. 111, 213-220. doi:10.1016/j.marpolbul.2016.07.006

Steensgaard, I.M., Syberg, K., **Rist, S.**, Boldrin, A., Hartmann, N., Hansen, S.F. 2017. From macro- to microplastics – Analysis of EU regulation along the life cycle of plastic bags. *Environmental Pollution*. 224, 289–299. doi:10.1016/j.envpol.2017.02.007

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This PhD has been a truly rewarding experience, both for my personal and professional development, and I feel deep gratitude towards everyone who supported and accompanied me on parts of this journey.

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These 3 years would not have been the same without the love and support of my family and friends. My deep gratitude goes to my mother, Raphael, Lisa, Therese, Pau, Myri, Roya, Christina and many more.
Thank you for always being there!

"*The more clearly we can focus our attention on the wonders and realities of the universe about us, the less taste we shall have for destruction*."
(Rachel Carson)

Summary

Within the past decade, it has been widely recognised that microplastics (commonly referred to as plastic particles <5 mm) are ubiquitous in freshwater as well as in marine environments globally. Owing to their small size, microplastics can interact with and potentially affect a wide range of aquatic organisms. Although the number of studies on microplastic effects is quickly increasing, there is still limited understanding of the processes by which organisms interact with microplastics as well as impacts in natural ecosystems. Further uncertainties relate to the chemical nature of microplastics and their potential role as vectors for chemical pollutants to organisms. More recently, questions have been raised about human exposure to microplastics and potential health effects – a topic where science still is at the very start of providing answers.

In this context, the aims of this thesis are: 1) To critically evaluate and use controlled laboratory experiments for analysing uptake and effects of microplastics in aquatic invertebrates. 2) To examine the interaction between plastic particles and hydrophobic organic chemicals. 3) To review the current debate and state of knowledge on microplastic exposure and potential effects on humans.

Most effects of microplastics on aquatic invertebrates have been studied as a result of particle ingestion. In order to understand and interpret such effects, it is important to quantify ingestion and egestion of microplastics, as this determines the overall exposure that an organism is facing. In this thesis, it is shown that fluorescent particles can be used to quantify these processes. This is especially useful for particles in the nano- and small micrometre size range. To achieve a reliable quantification, it is often necessary to digest animal tissue. Enzymes are recommended for digestion, based on the use and development of different enzymatic protocols within this thesis. Enzymatic digestion and quantification of particle fluorescence were successfully applied to measure ingestion and egestion of 100 nm and 2 µm particles in the water flea *Daphnia magna* and larvae of the blue mussel *Mytilus edulis*. For both species, it was found that, on a mass basis, ingestion of particles which are similar to the size of normal prey was by a factor 5 higher than of smaller particles. Regarding particle egestion, more species-specific differences were observed in comparison to ingestion. It was found that egestion can strongly be

influenced by particle size and the presence of food. Also, particles have the potential to remain in organisms for a time exceeding the normal gut passage time. Both for *D. magna* and larvae of *M. edulis* the smaller particles were found to cause more adverse effects on the animals' physiology, such as decreased feeding in daphnids and abnormal development in mussel larvae.

Controlled laboratory tests, as employed in this thesis, can be a useful tool to obtain a mechanistic understanding of organism-particle interactions and increase the reliability of and comparability between studies. It was, however, found that a detailed particle and exposure characterisation is often missing and thus particle behaviour and fate in laboratory exposure systems are not well understood. Drawing on experience and developments within the field of engineered nanomaterials, it is therefore recommended to include analyses of particle size, composition, density, surface chemistry and charge, as well as particle aggregation/agglomeration, dispersion and sedimentation. At the same time, it is important that exposure systems attain a higher degree of environmental realism. To achieve this, it is suggested to use lower particle concentrations, a variety of particle shapes (especially fragments and fibres), a variety of different plastic polymers, biofouled particles, and to include controls with natural particles, such as clay or silica.

Moreover, microplastics cannot always be treated as inert particles since they may contain a multitude of different chemicals, either stemming from plastic production or having sorbed to the plastics in the environment. In both cases, chemicals have the potential to get transported and released, and in this way microplastics may act as vectors for hydrophobic organic chemicals (HOCs) to aquatic animals. It is therefore strongly recommended to include controls for potential chemical toxicity in microplastic effect studies. As reviewed in this thesis, sorption of HOCs to plastics is governed by diffusive mass transfer and occurs as either adsorption, absorption or a combination of both. The process strongly depends on the properties of the plastic particle, the chemical and the surrounding environment. In comparison to natural matrices, such as water, dissolved organic carbon and colloids, the role of plastics as a vector may be negligible on a global scale. However, in this thesis it is emphasised that spatial variation on a smaller scale as well as the exposure route of microplastic-associated chemicals to organisms are important to consider.

In recent years, there has been an increasing focus on human exposure to and potential health effects of microplastics. This was mainly sparked by findings

of plastic particles in aquatic species used for human consumption as well as other food products, and has evoked many concerns. While there is reason to assume that microplastics can exhibit particle- and/or chemical-related toxicity, no studies have investigated human health effects of consuming microplastics to date. Humans are exposed to plastic particles and associated chemicals by a variety of pathways. Even though contaminated food products have received most attention, in this thesis it is argued that the main exposure is most likely related to abrasion of particles from the use of plastic materials in everyday life.

Because of many uncertainties and knowledge gaps, it is to date not possible to conclude to what degree microplastics are a threat to the environment and to humans. However, a strong public opinion against environmental plastic pollution has formed, which drives societal and legislative action. This is moving faster than consensus within the scientific community and thus entails the risk that not the most urgent issues are addressed or the most effective measures to reduce environmental plastic pollution are taken.

Dansk sammenfatning

I løbet af det seneste årti er man begyndt at erkende, at mikroplast (plastpartikler <5 mm) på globalt plan er allestedsnærværende i både hav- og ferskvandsmiljø. Som konsekvens af den lille størrelse kan mikroplast potentielt påvirke en lang række vandlevende organismer. Selvom antallet af studier omhandlende effekter af mikroplast er stærkt stigende, er forståelsen af processerne, hvormed organismer interagerer med mikroplast og de medfølgende indvirkninger på økosystemer, stadigvæk begrænset. Der er yderligere usikkerhed omkring mikroplastens kemiske sammensætning og dens potentielle rolle som vektor for kemikalier til organismer. På det seneste er der rejst opmærksomhed om den humane eksponering for mikroplast og mulige resulterende helbredseffekter – et emne hvor videnskaben stadig er i sin vorden.

I den sammenhæng er målene med denne afhandling: 1) At vurdere og anvende kontrollerede laboratorieforsøg til at analysere optag og effekter af mikroplast i hvirvelløse dyr i både hav- og ferksvandsmiljøer. 2) At undersøge interaktioner mellem plastpartikler og hydrofobe organiske kemikalier. 3) At gennemgå den aktuelle debat og tilgængelige viden indenfor human mikroplasteksponering og mulige humane effekter af denne.

De fleste undersøgelser af mikroplasts effekt på hvirvelløse dyr har været med fokus på effekter som følge af indtagelse. For at forstå og fortolke den slags effekter, er det vigtigt at kvantificere indtagelsen og udskillelsen af mikroplast, da disse processer er bestemmende for den overordnede eksponering. I denne afhandling påvises det, at fluorescerende partikler kan bruges til at kvantificere processerne, særligt når partiklerne er i størrelsesordenen af nanometer og få mikrometer. For en pålidelig kvantificering er det ofte nødvendigt at opløse dyrevæv. Baseret på anvendelse og udvikling af forskellige enzymatiske protokoller i denne afhandling, anbefales det at anvende enzymer til at opløse vævet. Enzymatisk opløsning og kvantificering af partikelfluorescens blev anvendt til at måle indtagelse og udskillelse af 100 nm og 2 µm partikler i vandloppen *Daphnia magna* og blåmuslingelarver (*Mytilus edulis*). For begge arter blev det påvist, at partikler med en størrelse svarende til dyrenes normale bytte blev optaget i en større grad end mindre partikler. Med hensyn til udskillelse fandtes flere artsspecifikke forskelle. Denne proces kan påvirkes markant af partikelstørrelsen og tilstedeværelsen af føde. Desuden kan partiklerne blive i organismen i længere tid end den tid, det normalt tager at passere gennem tarmsystemet. I både *D. magna* og larver af *M. edulis* havde de mindre partikler større negative effekter på dyrenes fysio-

logi såsom et lavere fodreindtag for vandlopperne og en unormal udvikling af blåmuslingelarverne.

Kontrollerede laboratorieforsøg, som anvendt i denne afhandling, kan forbedre den mekanistiske forståelse af organisme-partikel interaktionen og derved højne pålideligheden og øge sammenligneligheden mellem studier. En detaljeret karakterisering af partiklerne og eksponeringen mangler dog ofte og dermed mangler også en tilstrækkelig forståelse af partiklernes egenskaber og skæbne i det givne forsøg. På grundlag af erfaringer gjort i forbindelse med nanomaterialer anbefales det, at man også analyserer mikroplastpartiklers størrelse, sammensætning, massefylde, overfladekemi og elektriske ladning såvel som undersøger for mulig partikelaggregering, dispersion og sedimentation. Samtidig er det vigtigt, at eksponeringssystemer har en højere grad af miljørealisme. Det kan opnås ved at bruge lavere partikelkoncentrationer, flere forskellige partikelformer (især fragmenter og fibre), flere forskellige plastpolymere, partikler med biofilm og inkludere kontroller med naturlige partikler såsom ler og silikat.

Mikroplast kan ikke altid behandles som inerte partikler, for de kan indeholde mange forskellige kemikalier, som enten stammer fra plastproduktionen, eller er absorberet til plasten fra det omgivende miljø. I begge tilfælde kan kemikalierne transporteres og afgives, og på den måde kan mikroplast måske være en vektor for eksponering af vanddyr til hydrofobe organiske kemikalier. Derfor anbefales det på det kraftigste at inkludere undersøgelser af mikroplastpartiklernes potentielle kemiske toksicitet, når der udføres effektstudier af mikroplast. Som vist i denne afhandling, er sorption af hydrofobe organiske kemikalier til plast styret af diffusionsprocesser og kan optræde som absorption, adsorption eller en kombination af begge. Processen er afhængig af plastpartiklernes egenskaber, de hydrofobe kemikalier og det omgivende miljø. I naturlige matricer som havvand, opløst organisk kulstof og kolloider kan mikroplastens rolle som vektor muligvis være forsvindende lille (i særdeleshed på global skala). I denne afhandling understreges det dog, at rumlig variation på lokal skala samt eksponering af mikroplast-associerede kemikalier til organismer nødvendigvis må adresseres.

I de sidste år har der været et øget fokus på human eksponering og de mulige resulterende helbredseffekter. Bekymringen blev primært vakt på grund af fund af plastpartikler i vanddyr, som bruges til mad, og andre fødevarer. Der er grund til at antage, at mikroplast kan have partikel- eller kemikalierelateret toksicitet, men ingen studier har endnu undersøgt helbredseffekter

relateret til indtagelsen af mikroplast. Mennesker er eksponeret til mikoplast og associerede kemikalier fra en række forskellige kilder og selvom forurenede fødevarer har fået mest opmærksomhed, argumenteres der i denne afhandling for, at den største eksponering kommer fra brug af plastmaterialer i hverdagen.

På grund af mange usikkerheder og manglende viden er det ikke muligt endnu at konkludere i hvilken udstrækning mikoplast udgør en trussel for miljø og mennesker. Der er i dag en stærk folkestemning imod plastforurening i miljøet, som fremmer handlinger i samfund og lovgivning. Processen går hurtigere end konsensus i det videnskabelige miljø og dermed er der en risiko for, at fokus ikke er rettet mod mest pressende emner set fra et videnskabeligt synspunkt og der derved ikke bliver taget de mest effektive skridt for at begrænse plastforureningen i miljøet.

Table of contents

Abbreviations

BPA	Bisphenol A
EC_{50}	Median effect concentration
ENM	Engineered nanomaterial
HDPE	High density polyethylene
HOC	Hydrophobic organic chemical
LDPE	Low density polyethylene
NOM	Natural organic macromolecules
PA	Polyamide
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PE	Polyethylene
PEC	Predicted environmental concentration
PET	Polyethylene terephthalate
PHB	Polyhydroxybutyrate
$PM_{2.5}$	Airborne particulate matter <2.5 μm
PMMA	Poly(methyl methacrylate)
PNEC	Predicted no effect concentration
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinylchloride

1 Background and aims

It is widely documented and recognised that aquatic environments on a global scale are polluted with plastics. However, in the past decade the research focus as well as the public attention has increasingly shifted towards the smallest fraction of plastic debris in the size of a few millimetres and below, comprising particles that either enter the environment in this size or stem from the fragmentation of larger plastic debris. These so-called microplastics are commonly defined as plastic particles <5 mm (Arthur et al., 2009), which includes a wide variety of particles differing in composition, shape, size and origin. In the sub-micron size range, they are also referred to as nanoplastics (Gigault et al., 2018). However, in this thesis the term microplastics will be used for all particles <5 mm, including those in the nanometre size range. Worldwide analyses of water and sediment have shown that microplastics are ubiquitous in aquatic environments, including marine as well as freshwater systems (Duis and Coors, 2016).

Microplastics in the aquatic environment have the potential to affect a wide range of organisms since the small size of the particles makes them available to species of many different taxa. Accordingly, microplastic ingestion has been observed in a variety of organisms, including species of zooplankton (Cole et al., 2013), molluscs (Bour et al., 2018; Van Cauwenberghe et al., 2015), polychaetes (Browne et al., 2013) and echinoderms (Graham and Thompson, 2009). Adverse effects of microplastics on aquatic invertebrates have been found on different biological levels: from cells and tissues to whole organs and organisms (Galloway et al., 2017).

Still, the processes of particle ingestion – often a prerequisite for biological effects – as well as egestion are not well understood (Au et al., 2017). This is, however, crucial for evaluating the extent to which organisms are exposed to microplastics and interpreting biological effects. A further challenge is that most effect studies differ considerably in their design, using a variety of particle types, exposure concentrations, exposure durations, test species and biological endpoints. This hampers the comparability of results between studies as well as the reproducibility (Rist and Hartmann, 2018 - Paper III). Therefore, the **first aim** of this thesis is to critically evaluate and use controlled laboratory experiments for analysing uptake and effects of microplastics in aquatic invertebrates. More specifically, it is investigated in what quantities selected aquatic invertebrate species ingest and egest microplastics, and how this is influenced by different factors, such as particle size, exposure time and

food availability (addressed in Chapter 3 and in Paper I, IV and V). Furthermore, it is examined where in the organism the particles are located and whether the exposure affects their physiology and fitness (addressed in Chapter 3 and in Paper I, V and VI). As an approach to increase the comparability and reproducibility of microplastic effect studies, it is also evaluated to what extent standard procedures that have been developed in the laboratory work with engineered nanomaterials (ENMs) can be applied to the work with microplastics (addressed in Chapter 5 and in Paper III).

While most work on microplastics focuses on their physical nature, the chemical nature has the potential to affect organisms as well (Anbumani and Kakkar, 2018). This is important to consider since plastic particles have been associated with a variety of chemicals that are known to be hazardous to aquatic organisms (Cole et al., 2011). These chemicals are either part of the plastic material itself or they sorb to the plastic upon contact in the environment. To enhance the understanding of these processes, the **second aim** of this thesis is to examine the interaction between plastics and chemicals. Firstly, the mechanisms of sorption of hydrophobic organic chemicals (HOCs) to microplastics are studied. It is then discussed in what way microplastics can constitute a potential vector for chemicals to aquatic organisms (addressed in Chapter 4 and in Paper III and VII).

Findings of microplastics and plastic-associated chemicals in aquatic species used for human consumption as well as in individual food products have recently led to an increased focus on potential implications for human health (Barboza et al., 2018; Wright and Kelly, 2017). There is, however, a big discrepancy between the depiction and communication of risks related to consuming microplastics and actual scientific knowledge. The **third aim** of this thesis is therefore to review the current debate and state of knowledge regarding human exposure to microplastics as well as potential effects. Different exposure pathways of microplastics and associated chemicals are compared and the communication of health risks related to microplastics in food products is critically discussed (addressed in Chapter 6 and in Paper II).

2 Introduction

2.1 Plastic pollution in the aquatic environment

Since mass production of plastics started in the 1950s, global production volumes increased rapidly – a development that is continuing up to today (Geyer et al., 2017). The success of plastics, which has surpassed most other anthropogenic materials within a relatively short time span, is attributed to several unique properties, such as high durability, light weight, low cost and high adaptability for different applications (Andrady, 2011). This has resulted in substantial improvements in the sectors of human health, food security, transport and production costs, as well as giving rise to completely new products and applications (UNEP, 2018a). However, approximately 70% of all plastics ever produced, corresponding to 5,800 million tons, have become waste and an estimated 79% (or 4,900 million tons) of the plastic waste had accumulated in landfills or the environment by 2015 (Geyer et al., 2017). One of the main benefits of plastics – namely the high durability – thereby becomes a curse. The majority of plastics that have entered the environment are expected to still exist and it has even been proposed that plastics can be used as a key geological indicator of the Anthropocene (Zalasiewicz et al., 2016).

The worldwide pollution of aquatic environments with plastic waste is now acknowledged as a global problem. Of all litter, 60 to 80% is composed of plastics (Derraik, 2002) and for the year 2010 it was estimated that 4.8 to 12.7 million tons of plastics entered the oceans worldwide, a number that could increase by one order of magnitude until 2025 (Jambeck et al., 2015). In 2014, it was estimated that more than 5 trillion pieces of plastic weighing almost 269,000 tons were floating on the surface of the world's oceans (Eriksen et al., 2014). But this is only a small fraction of all plastics in the oceans as it has been reported that 99.8% of all plastics which had entered the marine environment since 1950 had settled below the surface by 2016 (Koelmans et al., 2017b). Most plastics enter the environment as macrodebris (so called macroplastics), but because of fragmentation and degradation processes ever smaller pieces and particles are formed (Andrady, 2011). In the last decade, the scientific focus increasingly shifted from macroplastics towards the smallest fraction of plastic debris in the size of a few millimetres and below: so called microplastics. Microplastics are at least as widespread in aquatic environments as macroplastics and on a number basis, microplastics

have even been found to constitute the majority of plastic debris in certain locations (Martins and Sobral, 2011).

2.2 Microplastics: definition and categories

Although the field of microplastic research has grown substantially within the past few years, no agreement on a clear definition of the term 'microplastics' has been reached yet. In 2004, the term was first used in the scientific literature to describe "microscopic plastic fragments and fibers" detected in marine samples (Thompson, 2004). Still, it took four more years until the first working definition was proposed, including all plastic particles <5 mm in the term (Arthur et al., 2009). While this definition is most frequently adopted in the field up to today, the use of 1 mm as the upper size limit is not uncommon (e.g. Andrady, 2015; Costa et al., 2010). There is no widespread consent regarding the lower size limit of microplastics. Resulting from the common use of neuston nets for sampling microplastics in water, the mesh size of 333 µm has been proposed as the lower size limit (Arthur et al., 2009). Mostly though, the term is used for particles with even smaller sizes (e.g. Cole et al., 2013; Paul-Pont et al., 2016). More recently, the term 'nanoplastics' emerged, for which the definition is even less clear, but which is mainly used for particles <1 µm or <100 nm (Gigault et al., 2018). In this thesis, the term microplastics is used to cover all plastic particles <5 mm, including those in the nanometre size range, to avoid confusion between different studies.

The use of different definitions entails several problems. It leads to ambiguous communication and makes results of different studies difficult or impossible to compare. Furthermore, clear definitions are essential in a regulatory context (Hartmann et al., 2018 - Paper VIII). Therefore, the scientific community should work towards a common definition and categorization framework. Hartmann et al. (2018 - Paper VIII) recently proposed a framework, which uses physico-chemical properties as defining criteria, while size, shape, colour and origin serve as classifiers (Fig. 1). As the term 'plastics' comprises a wide variety of different materials, a definition for environmental plastic debris needs to specify which materials it covers. This can be determined based on the chemical composition, solid state and solubility of the material (Fig. 1, criteria I, II, III). Hartmann et al. (2018 - Paper VIII) suggest to define environmental plastic debris as synthetic or heavily modified natural polymers that, when present in natural environments, are solid and insoluble at 20°C. It is obvious that the most common synthetic polymers that are

reported in microplastic samples from the aquatic environment fall under the term 'plastics'. These include low and high density polyethylene (LDPE, HDPE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) (Andrady, 2011). However, in other cases, such as paints, tyre wear particles and polymer gels, it is not clear whether they should be considered as plastics. Such special cases need to be specified separately (Hartmann et al., 2018 - Paper VIII).

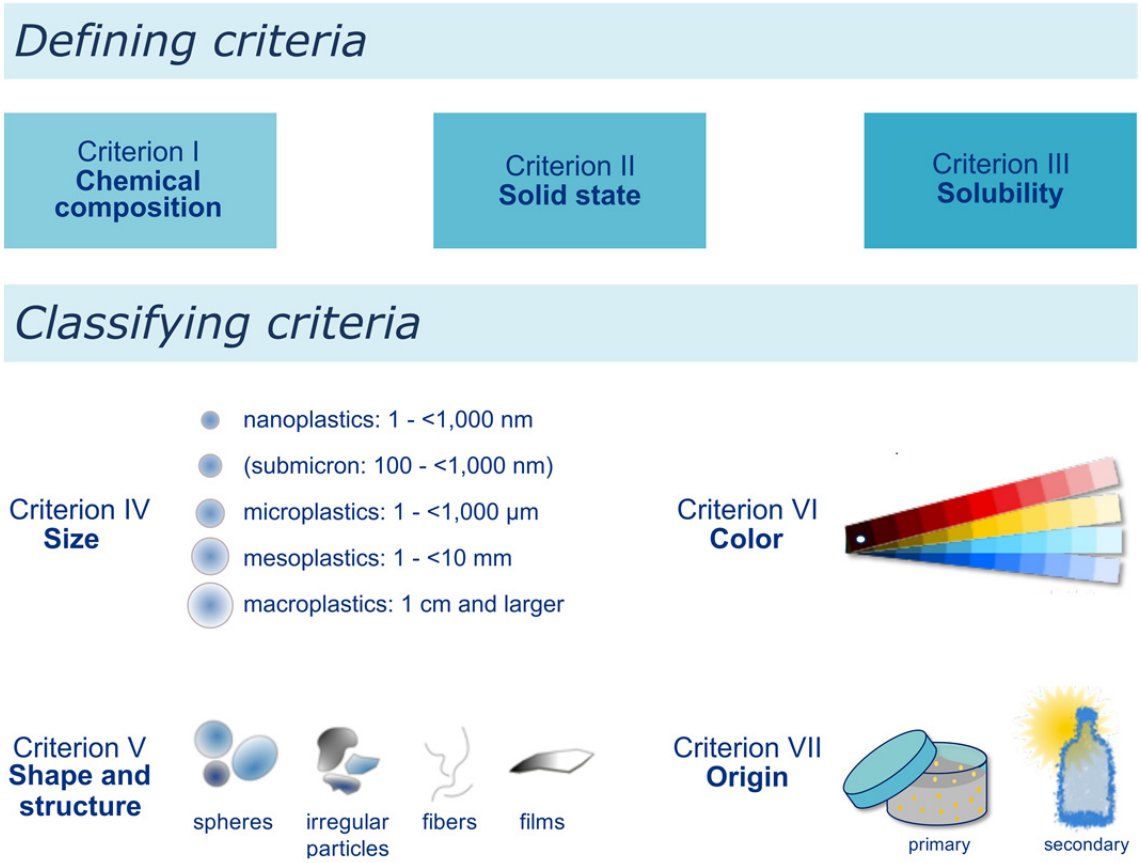

Figure 1. A definition and categorization framework for plastics as proposed by Hartmann et al. (2018 - Paper VIII). Figure modified from Hartmann et al. (2018 - Paper VIII).

Concerning particle sizes, the framework suggests the following ranges: nanoplastics 1 to <1,000 nm (with the subgroup of submicron-plastics 100 to <1,000 nm), microplastics 1 to <1,000 µm, mesoplastics 1 to <10 mm and macroplastics 1 cm and larger (Fig. 1, criterion IV). In addition to size, commonly used criteria for microplastics are particle shape, colour and origin (Hidalgo-Ruz et al., 2012), which have also been adopted in the framework by Hartmann et al. (2018 - Paper VIII). Particle shapes are proposed to be classified as spheres, irregular particles, fibres and films (Fig. 1, criterion V).

To reduce the bias by the person analysing the sample, the colour should be evaluated based on standardized colour palettes (Fig. 1, criterion VI). Particle origin, mainly of microplastics, is usually divided into primary and secondary. Primary microplastics are intentionally produced in this size range, while secondary microplastics result from the fragmentation of bigger plastic items (Cole et al., 2011; GESAMP, 2015). The term primary microplastics is sometimes also used for particles that have been formed by fragmentation resulting from human activity before entering the environment, such as textile fibres and tyre wear particles, whereby secondary microplastics only refers to particles that have fragmented in the environment (Boucher and Friot, 2017; MEPEX, 2014). However, once they are in the environment, it is almost impossible to determine whether a particle has fragmented before or after entering it. Therefore, Hartmann et al. (2018 - Paper VIII) proposed to use the former definition of the term (Fig. 1, criterion VII).

The research field of microplastics is developing quickly and in order for a definition and categorisation system to be widely adopted, there must be broad consensus within the scientific and regulatory community. Therefore, the framework by Hartmann et al. (2018 - Paper VIII) is not meant to provide a set definition but rather a starting point for consensus-building.

2.3 Sources and distribution of microplastics

The majority of microplastics in aquatic environments are of secondary origin, with mismanaged waste being the main source (Andrady, 2011; UNEP, 2018b). Further considerable sources are synthetic fibres released during the washing of textiles and particles that are abraded from tyres (Napper and Thompson, 2016; Wagner et al., 2018). In the environment, secondary microplastics are not only formed by fragmentation due to physical impacts (e.g. action of waves, rocks and sand). Plastic degradation also plays an important role as the degradation results in a more brittle material that can fragment easily. The main degradation processes in the environment are photo- and thermooxidative degradation. The extent to which these take place depends on the light, temperature and oxygen conditions at a specific location (Andrady, 2011). Sources of primary microplastics are pre-production pellets and plastic particles used as abrasives in cosmetics and washing products, as well as in sand blasting machines (Fendall and Sewell, 2009; Gregory, 1996).

There is a variety of pathways by which macro- and microplastics enter the environment, including wind-blown transport, outlets of waste water treatment plants, road-runoff, spills and direct littering (Duis and Coors, 2016). Once in the aquatic environment, floating plastics are very mobile: they get transported from freshwater systems to the sea by rivers, and ocean currents further distribute them globally. Accordingly, microplastics have been found in aquatic ecosystems worldwide, including remote areas, such as uninhabited islands (Lavers and Bond, 2017), the Arctic (Peeken et al., 2018) and Antarctica (Isobe et al., 2017; Peeken et al., 2018).

The fate of microplastics in aquatic environments is strongly influenced by the particle properties, such as density, shape and size. For instance, the density determines whether a particle floats on the water surface or sinks (Andrady, 2011). This depends on the material composition of the particle but also on the state of degradation and biofouling, which can substantially increase the density so that also positively buoyant plastics (e.g. PE) finally sink. It is therefore expected that sediments are a major sink for microplastics (Woodall et al., 2014). However, biofouling and subsequent sinking of plastic particles is a reversible process, meaning that a particle's position in the water column will likely vary over time (Ye and Andrady, 1991).

Even though microplastics are ubiquitous, their abundance in the aquatic environment is very heterogeneous. Accordingly, a wide range of concentrations have been reported that differ by several orders of magnitude (Table 1). For instance, up to 6.8 items m^{-3} (particles >100 µm) have been reported in surface water samples in the Baltic Sea (Setälä et al., 2016a). In Jinhae Bay, South Korea, a maximum of 247,000 plastic pieces m^{-3} was found, which however also covered particles <100 µm (Song et al., 2015). Generally, microplastic concentrations are elevated along coastlines (Cole et al., 2011), especially close to urban areas (Browne et al., 2011; Leite et al., 2014). Also the big ocean gyres are known hotspots of plastic litter (Cozar et al., 2014). Several models have been developed to describe global microplastic distribution in the oceans (Cozar et al., 2014; Eriksen et al., 2014; van Sebille et al., 2015). However, they come with a high degree of uncertainty due to a lack of data for many areas (van Sebille et al., 2015). Even less data is available for freshwater environments, but measured microplastic particle numbers indicate that the degree of pollution is comparable (Li et al., 2018).

Table 1. Numbers of microplastics that have been detected in water and sediment samples in the marine and freshwater environment at different locations globally

Location	Lower size limit	Sampling method	Microplastic particle number	Reference
Water samples marine				
Baltic Sea	>100 µm	Pump	0-6.8 m^{-3}	Setälä et al., 2016a
Jinhae Bay, South Korea	n.d. (50 µm)	Sea surface microlayer	88 ± 68 L^{-1}	Song et al., 2015
NE Atlantic	>250 µm	Pump	2.46 ± 2.43 m^{-3}	Lusher et al., 2014
North Pacific Subtropical Gyre	>335 µm	Neuston net	0-1.2·10^7 km^{-2}	Law et al., 2014
Ross Sea, Antarctica	>5.5 µm	Pump	0.0032-1.18 m^{-3}	Cincinelli et al., 2017
Sediment samples marine				
North coast of Taiwan	>38 µm	Beach sampling	320-42,560 m^{-3}	Kunz et al., 2016
North Sea coast, Netherlands	>10 µm	Van Veen grab	100-3,600 kg^{-1}	Leslie et al., 2017
South coast of Tuscany, Italy	>63 µm	Beach sampling	42-1,069 kg^{-1}	Cannas et al., 2017
Wadden Sea, Germany	n.d. (100 µm)	Beach sampling	0-62,100 kg^{-1}	Liebezeit and Dubaish, 2012
Water samples freshwater				
Amsterdam canal, Netherlands	>10 µm	Surface water in glass bottles	48-187 L^{-1}	Leslie et al., 2017
Lake Erie, USA	>333 µm	Manta trawl	4,686-466,305 km^{-2}	Eriksen et al., 2013
Lake Geneva, Switzerland	>300 µm	Manta trawl	11,000-220,000 km^{-2}	Faure et al., 2015
Lake Hovsgol, Mongolia	>333 µm	Manta trawl	997-44,435 km^{-2}	Free et al., 2014
River Rhine, Germany	>300 µm	Manta trawl	52,364-3.9·10^6 km^{-2}	Mani et al., 2016
Sediment samples freshwater				
Amsterdam canal, Netherlands	>10 µm	Van Veen grab	<68-10,500 kg^{-1}	Leslie et al., 2017
Edgbaston Pool, UK	>500 µm	HTH gravity corer	0-260 kg^{-1}	Vaughan et al., 2017
Lake Geneva, Switzerland	>300 µm	Beach sampling	78-5,000 m^{-2}	Faure et al., 2015
St. Lawrence River, Canada	>500 µm	Petite Ponar grab	0-136,926 m^{-2}	Castañeda et al., 2014

n.d.: no data on the lower size limit given, size in brackets is the smallest reported particle size

A major issue regarding microplastic abundance is that the methods for sampling, sample treatment, analysis and data reporting are not standardised, which can greatly hamper the comparability between different studies (Miller et al., 2017). Reported abundances depend on, for example, the sampled size range and particle types (fibres are included in some studies but excluded in others). Furthermore, it can be expected that concentrations of microplastics in the field are currently largely underestimated as the lower size limit when using neuston nets usually is 333 µm. Even when aiming for smaller fractions, it is because of technical limitations hardly possible to obtain reliable results for particles <10 µm (Shim et al., 2017). It therefore remains largely unknown whether and to what degree particles in the nanometre and small micrometre size range can be found in the environment. However, their presence has recently been demonstrated in the North Atlantic Subtropical Gyre (Ter Halle et al., 2017). This is an important aspect as increasing particle numbers have been observed for decreasing size classes during the fragmentation of plastics (Lambert and Wagner, 2016) as well as in environmental samples (Song et al., 2015). It may therefore be assumed that the majority of plastic particles in the environment are of sub-micron size.

2.4 Interactions of microplastics with animals and humans

It is well known that macroplastic litter constitutes a threat to aquatic life, mainly due to entanglement and ingestion (Derraik, 2002). Several hundred species have been reported to encounter marine litter, with plastics accounting for 92% of the cases (Gall and Thompson, 2015). Also microplastics have the potential to affect a wide range of organisms. More than 220 species have been found to ingest microplastics in natural ecosystems (Lusher et al., 2017) and in some invertebrate species ingestion was observed in a majority (up to >80%) of the population (Devriese et al., 2015; Murray and Cowie, 2011). Attributable to their small sizes, microplastics are available to a large variety of organisms of different sizes, feeding strategies, trophic levels, and taxa. This includes zooplankton (Cole et al., 2013), bigger crustaceans (Watts et al., 2014), molluscs (Bour et al., 2018; Van Cauwenberghe et al., 2015), polychaetes (Browne et al., 2013), echinoderms (Graham and Thompson, 2009), fish (Foekema et al., 2013; Sanchez et al., 2014), marine mammals (Besseling et al., 2015) and seabirds (Mallory, 2008). Plastic particles are either ingested passively together with normal food and drinking, or they are mistaken for

food and actively fed on (Wright et al., 2013b). Ingestion is, however, not the only uptake route. Microplastics can enter organisms via the gills by ventilation as it has been observed in the shore crab *Carcinus maenas* (Watts et al., 2014). In the blue mussel *Mytilus edulis*, particle adherence to the soft tissue was described as a new way of uptake (Kolandhasamy et al., 2018). Furthermore, in the water flea *Daphnia magna* nano-sized particles were reported to enter the embryo via the mother's brood pouch (Brun et al., 2017).

It has been observed that some organisms can simply egest plastic particles again after ingestion, without any detectable effects (Bruck and Ford, 2018; Hämer et al., 2014). This supports the assumption that microplastics are sometimes comparable to natural suspended solids. However, other studies have reported effects of microplastic exposure on different levels of biological organisation, including gene expression, cellular and tissue function, physiology and survival (Canesi et al., 2015; Cole et al., 2015; von Moos et al., 2012; Rist et al., 2016, 2017, 2018a - Paper I, V) (see Chapter 3). It is also important to consider the chemical composition of microplastics, which is extremely variable and differs fundamentally from natural suspended solids. Chemicals have been shown to leach from plastics and impair aquatic organisms (Gandara e Silva et al., 2016; Nobre et al., 2015). Furthermore, translocation of plastic particles from the gastrointestinal tract to other tissues has been reported (Browne et al., 2008; Farrell and Nelson, 2013; Magni et al., 2018). This most likely prolongs their prevalence in an organism and increases the likelihood for effects. It also enhances the potential for trophic transfer of microplastics in the food web – a process that has been demonstrated in laboratory experiments as well as in the field (Farrell and Nelson, 2013; Nelms et al., 2018; Welden et al., 2018). However, it remains unclear whether a biomagnification of microplastics takes place from one trophic level to the next. The observations of trophic transfer in the aquatic food web along with the presence of plastic particles in several species for human consumption have triggered questions on possible implications for human health (Rist et al., 2018b - Paper II), which is discussed in Chapter 6.

3 Laboratory uptake and effect studies

3.1 Quantification of microplastics in organisms

In most cases, particle ingestion, or uptake by a different route such as ventilation, is a prerequisite for biological effects (Anbumani and Kakkar, 2018). In order to understand and evaluate observed effects, it is therefore important to quantify ingestion and egestion processes, and examine governing factors. However, the amount of plastic particles that is ingested and egested by an organism is not frequently quantified in microplastic exposure studies. Furthermore, the factors that influence these processes as well as the residence time of particles in organisms are not well understood (Au et al., 2017).

Quantifying microplastics in an organism is analytically challenging, even in laboratory studies with known particles. One has to deal with the complex matrix of animal tissues and visualization techniques for counting are limited by particle size and number. Most commonly, analysis is done on the whole organism or on the digestive tract after dissection (Lusher et al., 2017). Depending on the particle size, different microscopy methods can be applied: spanning from stereomicroscopy for the biggest size fraction to confocal microscopy for the small micrometre size range and electron microscopy for sub-micron sized particles.

If an organism is transparent, it is possible to observe plastic particles directly in the intact tissue. However, it can be difficult to count particles since they might lie on top of each other. In that case and if animals are not transparent, it is necessary to digest the tissue to retrieve the particles for counting. Different protocols have been used for digesting animal tissues, including acids (e.g. Claessens et al., 2013), bases (e.g. Foekema et al., 2013), hydrogen peroxide as an oxidising agent (e.g. Li et al., 2015; Mathalon and Hill, 2014) and enzymes (e.g. Cole et al., 2014; Courtene-Jones et al., 2017). Since enzymes have been shown to have minimal effects on plastic particles – an essential requirement for obtaining reliable results – while exhibiting high digestion efficiencies, their use has been suggested over chemical digestion agents (Cole et al., 2014; Karlsson et al., 2017; Railo et al., 2018). Especially industrial proteases have been recommended as they are mostly supplied in liquid form, making them safe to handle, and they are relatively inexpensive (Catarino et al., 2017; Rist et al., 2018c - Paper IV). Alcalase (an industrial protease supplied by Novozymes®) has recently been shown to digest whole soft tissues of *M. edulis* with an efficiency of >98% (Rist et al., 2018c -

Paper IV). The protocol is easy to handle with only one treatment step and does not require any additional chemicals. It was employed to retrieve 50 µm PS beads, which blue mussels had been exposed to in a laboratory experiment. Subsequently, the beads could be counted with a stereomicroscope (Rist et al., 2018c - Paper IV).

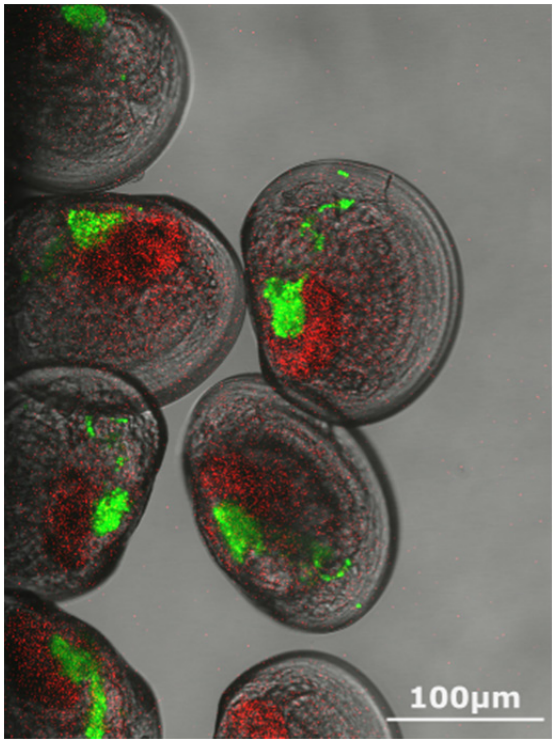

Figure 2. Confocal microscopy picture of blue mussel (*Mytilus edulis*) larvae after exposure to 2 µm fluorescent polystyrene beads (green). Figure from Rist et al. (2018a - Paper V).

For particles in the nanometre and small micrometre size range it is difficult or even impossible to count individual particles visually. Here, fluorescence can be used as a tool. Fluorescence labelling of particles can help to visualize them by fluorescence microscopy (Fig. 2). Furthermore, fluorescence intensity can serve as a proxy for particle mass or number. This was used by Rosenkranz et al. (2009) to quantify ingestion and egestion of 20 nm and 1 µm PS beads in *D. magna*. Similarly, Rist et al. (2017 - Paper I) determined the ingested and egested mass of 100 nm and 2 µm PS beads in *D. magna*. However, the total mass of ingested particles differed considerably between the studies. The measured values of 0.15 µg of the 100 nm and 0.75 µg of the 2 µm beads per animal after 4h of exposure in the study by Rist et al. (2017 - Paper I) were 2,500 and 500 times higher than of the 20 nm and 1 µm beads, respectively, reported by Rosenkranz et al. (2009). While the numbers are not directly comparable since different particle sizes and exposure concentrations were used, this difference may also be attributed to the sample treatment. Rist et al. (2017 - Paper I) enzymatically digested the daphnid tissues before measuring fluorescence intensity, whereas Rosenkranz et al. (2009) only mechanically homogenised the animals. The latter could lead to an underestimation due to particles remaining in the tissue. It is therefore vital to carefully choose and evaluate the quantification technique to obtain reliable results.

3.2 Factors influencing microplastic ingestion and egestion

The processes of microplastic ingestion and egestion – and with this the particle mass in an organism – can be influenced by a number of different factors, including particle concentration, size, shape, biofouling, exposure duration, food availability and the characteristics of the organism.

The concentration of microplastics in the water is the most obvious factor to influence particle ingestion. A direct positive relationship between the two can especially be expected for organisms that feed non-selectively. The more particles, the higher is the encounter rate with the organism and subsequently the particle ingestion rate. Accordingly, *M. edulis* ingested more PS beads at a higher in comparison to a lower concentration (100 and 5 beads L^{-1}, respectively) (Rist et al., 2018c - Paper IV). The increase of beads per animal was proportional to the increase of the exposure concentration, which indicates a passive ingestion of the beads. Similarly, Scherer et al. (2017) found increasing numbers of plastic beads per animal when exposing *D. magna*, *Chironomus riparius* larvae, *Gammarus pulex* and *Physella acuta* to increasing concentrations. The same tendency was also observed for the ascidian *Ciona robusta* (Messinetti et al., 2018) and larvae of the sea urchin *Tripneustes gratilla* (Kaposi et al., 2014). If, however, an organism selectively feeds on plastic particles or actively avoids them, the relationship between particle concentration in the water and the amount of ingested particles will differ.

When comparing two sizes of PS beads, both Rosenkranz et al. (2009) and Rist et al. (2017 - Paper I) found *D. magna* to ingest more of the bigger particles (on a mass basis) at the same mass concentration in the water. For instance, 2 µm beads were ingested by a factor 5 more in comparison to 100 nm beads, as depicted in Fig. 3 (Rist et al., 2017 - Paper I). Using the same quantification method, differential ingestion of 100 nm and 2 µm beads was also observed in a marine planktonic invertebrate: the larvae of the blue mussel (Rist et al., 2018a - Paper V). Although the particle mass per animal was much lower than what was found in the daphnids, due to the difference in body size, a similar relationship between both particle sizes was found with a mass difference of a factor 5 approximately. Particle egestion was independent of particle size in blue mussel larvae (Rist et al., 2018a - Paper V). However, for *D. magna* an influence of particle size was also found on egestion. This was more efficient for micrometre- than nanometre-sized beads (Rosenkranz et al., 2009; Rist et al., 2017 - Paper I). While Rosenkranz et al.

(2009), however, reported egestion of 40% for 20 nm beads and 90% for 1 µm beads within 4h, Rist et al. (2017 - Paper I) found rather high, constant masses of particles in the animals even after 24h of depuration, which only decreased by 29 and 36% for 100 nm and 2 µm beads, respectively (Fig. 3). Overall, the results show that particle size is a decisive factor in the interaction of organisms with micro- and nanoplastics, and it can be expected that particles of a similar size as an organism's prey are ingested in the highest quantities. Plastic particles that are far below the minimum prey size can still be ingested though or enter the gastrointestinal tract together with water.

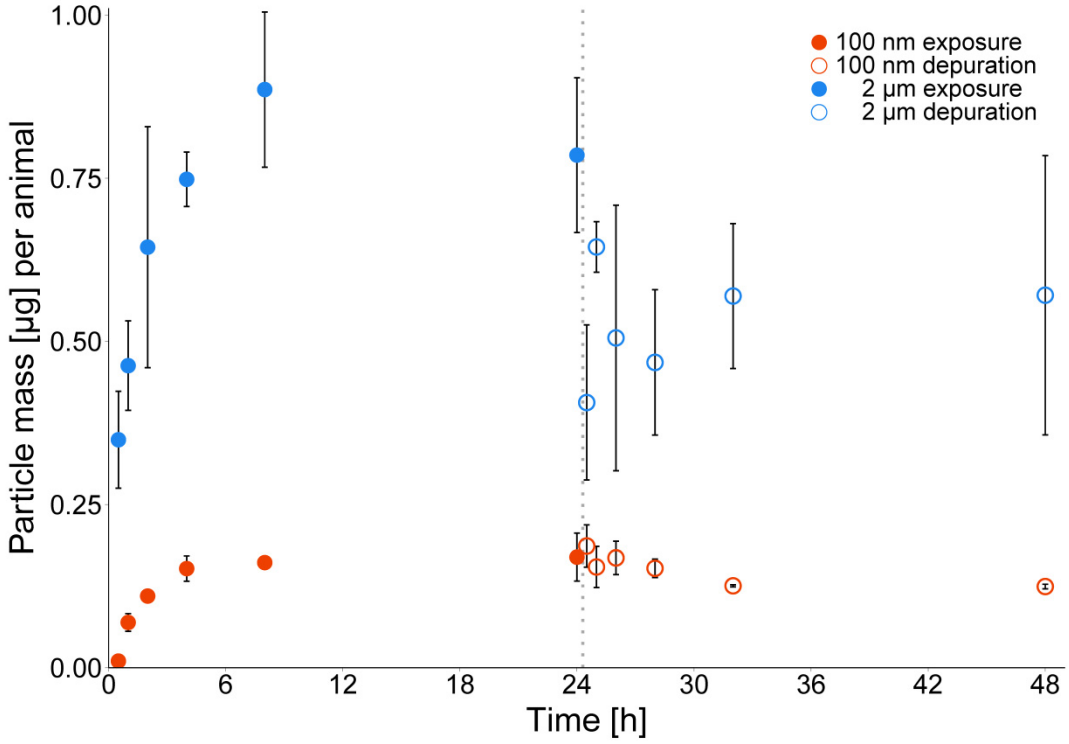

Figure 3. Mass of 100 nm and 2 µm polystyrene beads in *Daphnia magna* during 24h of exposure to a particle concentration of 1 mg L^{-1} and a subsequent 24h depuration period in clean water. Figure modified from Rist et al. (2017 - Paper I).

Most microplastic exposure studies use spherical particles since they are available in defined sizes and with different properties, such as fluorescent labelling or surface modifications. It has, however, been shown that particle shape can influence the degree of ingestion. In a comparison of spheres, fragments and fibres, Gray and Weinstein (2017) found significant differences between the ingested amounts of the three shapes in daggerblade grass

shrimp (*Palaemonetes pugio*), where fragments were ingested most and fibres least. No difference was, however, observed in the residence time of particles. This is in accordance with results by Vroom et al. (2017), who found a similar gut passage time for beads and fragments in the copepod *Calanus finmarchicus*. However, significantly slower egestion rates of plastic fragments in comparison to beads were observed in *D. magna* (Frydkjær et al., 2017). Also, in the freshwater amphipod *Hyalella azteca* PP fibres were egested 2 to 4 times slower than normal food and PE particles (Au et al., 2015). It can thus be concluded that ingestion as well as egestion can differ depending on the particle shape.

Another factor to consider is that microplastic particles change over time due to different processes. This includes biofouling, caused by the interactions with microorganisms, as well as changes in the physical/chemical properties of the plastic resulting from degradation and fragmentation. In copepods (*C. finmarchicus*) and Mediterranean mussels (*Mytilus galloprovincialis*), exposure of plastic particles to natural seawater for 3 weeks resulted in enhanced ingestion in comparison to virgin particles (Bråte et al., 2018; Vroom et al., 2017). This was attributed to the formation of a biofilm on the particle surface, which increases the resemblance to natural food items. Biofilms form rapidly on plastic particles in the aquatic environment and it can therefore be expected that the vast majority of microplastics that aquatic animals encounter are covered with a biofilm (Lobelle and Cunliffe, 2011; Rummel et al., 2017). To increase the understanding of how this influences ingestion and egestion processes, biofouling of particles should be included more frequently in exposure studies.

The time scales for ingestion and egestion processes are different depending on the organism. Thus, the duration of exposure and depuration phases should be chosen to meaningfully reflect the biology of the test species. The choice will influence what particle mass is detected in an organism. As depicted in Fig. 3, measuring the ingested amount of PS beads in *D. magna* in the course of 24h showed that the particle mass increased in the first 8h before a plateau was reached (Rist et al., 2017 - Paper I). Repeated quantification during a laboratory study provides most information. When only a single time point is measured, it should be considered that this value may be influenced by many different factors. Often, it is not straightforward to explain observations as in the case of particle egestion by *M. edulis* larvae, which showed a higher remaining particle mass in the animals after 48h than 16h of depuration (Rist et al., 2018a - Paper V). This was attributed to a possible re-ingestion of parti-

cles or to the use of mussels from two different populations. Measuring more time points in the course of the experiment to determine an ingestion rate for particles could have elucidated this surprising observation. Choosing single time points makes most sense when it can be expected that a steady state of particle mass in the organism is reached at the time of sampling. To design an experiment that can reliably measure steady states, it is essential to consider the time scales for ingestion and egestion processes in the specific test organism.

Whether or not food is provided during an exposure test can affect microplastic ingestion and egestion substantially. Both processes have been shown to be strongly influenced by the presence of food algae in *D. magna* (Aljaibachi and Callaghan, 2018; Scherer et al., 2017; Rist et al., 2017 - Paper I). When food was added during the egestion phase following exposure to 100 nm and 2 µm PS beads, almost complete egestion of both particle sizes was observed, in contrast to no significant reduction in the animals' particle mass in the absence of food (Rist et al., 2017 - Paper I). During exposure to the particles *D. magna* ingested 78 and 98% less of the 100 nm and 2 µm PS beads, respectively, in the presence of food in comparison to its absence (Rist et al., 2017 - Paper I). Similar trends were also observed by Scherer et al. (2017) and Aljaibachi and Callaghan (2018). This can partly be explained by a dilution effect since a substantial fraction of all particles is composed of algae instead of only plastics, resulting in less ingestion of the latter. Partly, however, it may also be attributed to selective feeding. Therefore, it is important to consider the potential influence of food availability in exposure tests.

Even though studies have in some cases found similar trends for microplastic ingestion and egestion in different organisms, the characteristics of the studied organism play an important role. This refers to the feeding mode and the species. Within one species, there are differences related to the animal size, age, developmental stage and possibly the population (Liu et al., 2018; Scherer et al., 2017; Setälä et al., 2016b; Vroom et al., 2017; Welden and Cowie, 2016a; Rist et al., 2018a - Paper V). It can therefore be challenging to draw general conclusions for a wider range of animals from the observations in individual organisms.

3.3 Localisation of microplastics within organisms

Most studies report microplastics in the gastrointestinal tract of organisms since particle ingestion is the uptake route that is mainly studied (e.g. Au et al., 2015; Bergami et al., 2016; Hämer et al., 2014). Other uptake routes result in a different localisation of particles. For instance, in the crabs *C. maenas* and *Uca rapax* microplastics were detected on the gills resulting from ventilation (Brennecke et al., 2015; Watts et al., 2014) and in *M. edulis* particles were observed to adhere to different organs of the soft tissue, which was suggested to constitute an uptake route beyond ingestion (Kolandhasamy et al., 2018).

In some species, translocation of plastic particles from the gastrointestinal tract to other tissues has been observed. In *M. edulis* 3 and 9.6 µm PS beads were detected in the circulatory system, where they persisted for more than 48 days (Browne et al., 2008), and even HDPE particles of up to 80 µm were reported to be taken up by lysosomes (von Moos et al., 2012). Similarly, in zebra mussels (*Dreissena polymorpha*) 1 and 10 µm PS beads penetrated the epithelium of the gastrointestinal tract and reached the circulatory system (Magni et al., 2018). Particle translocation to the circulatory system as well as other organs was observed in *C. maenas* during exposure to 500 nm PS beads (Farrell and Nelson, 2013). Nanometre-sized PS beads have also been reported to translocate to different organs in *D. magna* and *Daphnia galeata*, including lipid droplets, ovaries and embryos (Brun et al., 2017; Cui et al., 2017; Rosenkranz et al., 2009). The main method that is used for studying particle translocation is microscopic imaging of fluorescent particles (Triebskorn et al., 2018). However, using particle fluorescence for localisation entails a risk for experimental artefacts and misinterpretation. In an attempt to reproduce the findings by Rosenkranz et al. (2009), who reported translocation of 20 nm and 1 µm PS beads to the lipid droplets of *D. magna* at an exposure concentration of 2 µg L^{-1}, Schür et al. (2018 - Paper VI) could not detect particles outside the gastrointestinal tract. Only at a 1,000-fold increased concentration (i.e. 2 mg L^{-1}), fluorescence within the lipid droplets was observed for both particle sizes. However, it was shown that the signal was caused by the fluorescent dye alone that had detached from the particles (Fig. 4). Including appropriate controls is therefore important to ensure the stability of the particle labelling.

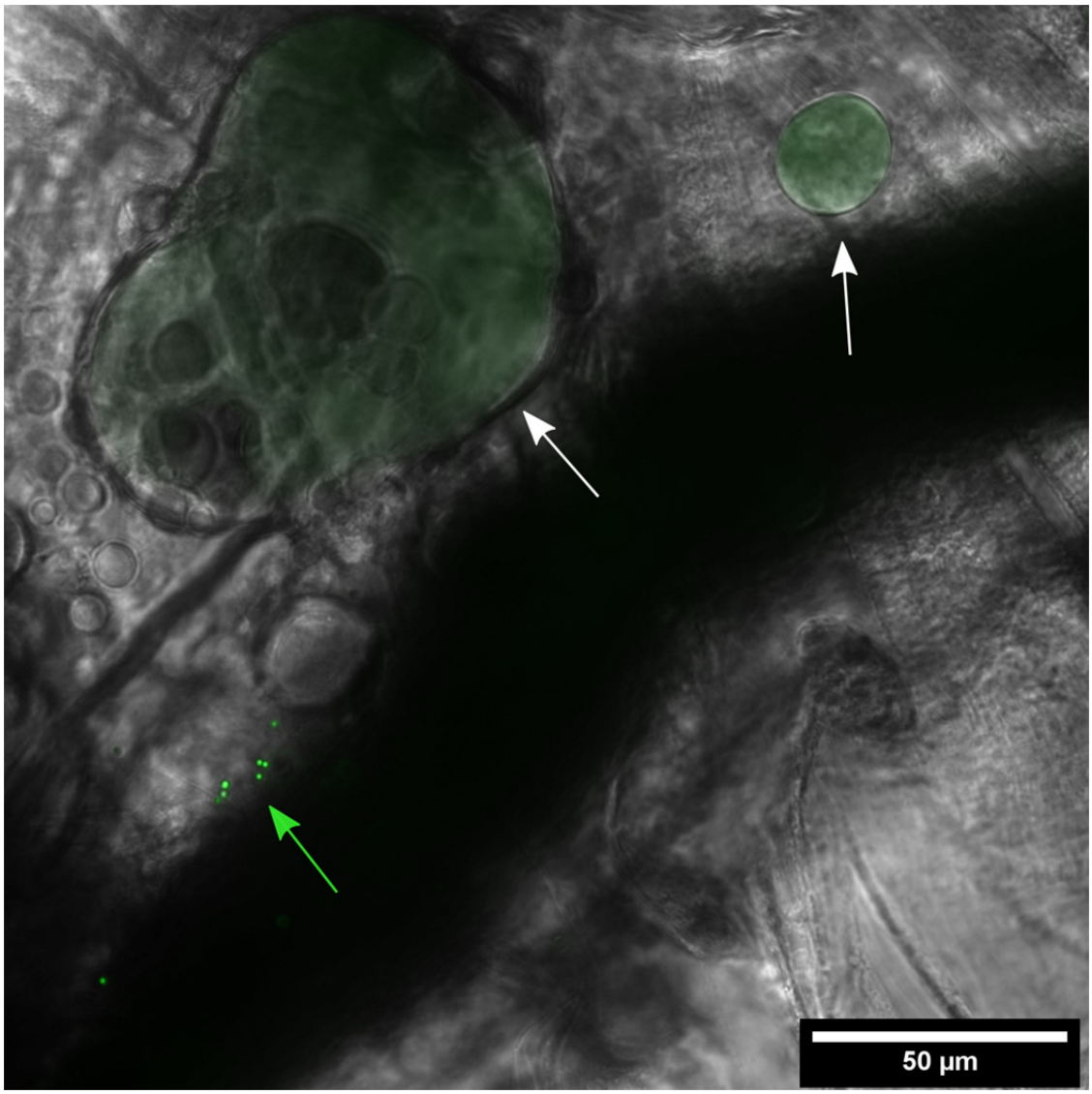

Figure 4. Confocal microscopy of the gastrointestinal tract of *Daphnia magna* after exposure to 1 µm fluorescent polystyrene beads. In the lipid droplets (white arrows) diffuse fluorescence was seen without visible particles. The latter were only observed in vicinity of the gastrointestinal tract (green arrow). This indicates that only the fluorescent dye and not the plastic particles had moved into the lipid droplets. Figure modified from Schür et al. (2018 - Paper VI).

Particle size is a major factor determining microplastic translocation, which is more plausible for particles in the nanometre size range as these may passively pass cell membranes or enter cells by endocytosis (Zhu et al., 2013). However, persorption may be a mechanism enabling translocation of particles up to 150 µm (Volkheimer, 2001). Also the surface chemistry most likely plays an important role (Triebskorn et al., 2018). This influences the interaction of particles with biological membranes. Positively charged plastic parti-

cles are more prone to adhering to, or perturbing, negatively charged lipid membranes. Accordingly, amino-modified cationic PS beads have been found to exhibit much higher toxicity in comparison to neutrally charged or anionic beads (Manfra et al., 2017; Tallec et al., 2018).

Microplastic particles that have translocated away from the point of initial uptake, mostly the gastrointestinal tract, to other organs and tissues cannot be excreted as easily and will most likely stay in the organism for a prolonged period of time (Anbumani and Kakkar, 2018). This may increase the chance for trophic transfer of microplastics. Furthermore, there is a higher likelihood for adverse effects on a cellular and/or physiological level as the presence of microplastics in tissues has been associated with oxidative stress and inflammatory responses (Avio et al., 2015; Paul-Pont et al., 2016; von Moos et al., 2012). Such translocation-related effects are more likely to occur for particles in the nano- than micrometre size range.

3.4 Physiological effects on aquatic invertebrates

A major question within the issue of environmental microplastic pollution is whether microplastics give rise to biological effects. Many studies have reported adverse effects of microplastics on marine and freshwater invertebrates, spanning across different levels of biological organisation (Fig. 5): from cells and tissues to whole organisms and populations (Galloway et al., 2017). There is, however, an increasing number of studies that report no effects (Beiras et al., 2018; Bruck and Ford, 2018; Hämer et al., 2014; Malinich et al., 2018; Santana et al., 2018). In a meta-analysis of the scientific literature regarding microplastic effects on feeding, growth, reproduction and survival in aquatic invertebrates and fish, Foley et al. (2018) found no measurable effects in the majority of cases, although there were adverse effects for every category. The variation between taxa was generally high, making it difficult to find common trends. However, the most consistent effect across taxa was seen on feeding. Furthermore, zooplankton was identified as the most susceptible group towards microplastic exposure.

It has been pointed out that the potential cause of effects generally depends on two factors: the physical and the chemical nature of the particles (Anbumani and Kakkar, 2018). Most microplastic research focuses on the former, which is covered in the current chapter. However, it is important to consider the chemical composition of microplastics as well since chemicals

of the plastic material itself, or those sorbed to the particles in the environment, may be responsible for observed effects on organisms. These aspects are discussed in Chapter 4.

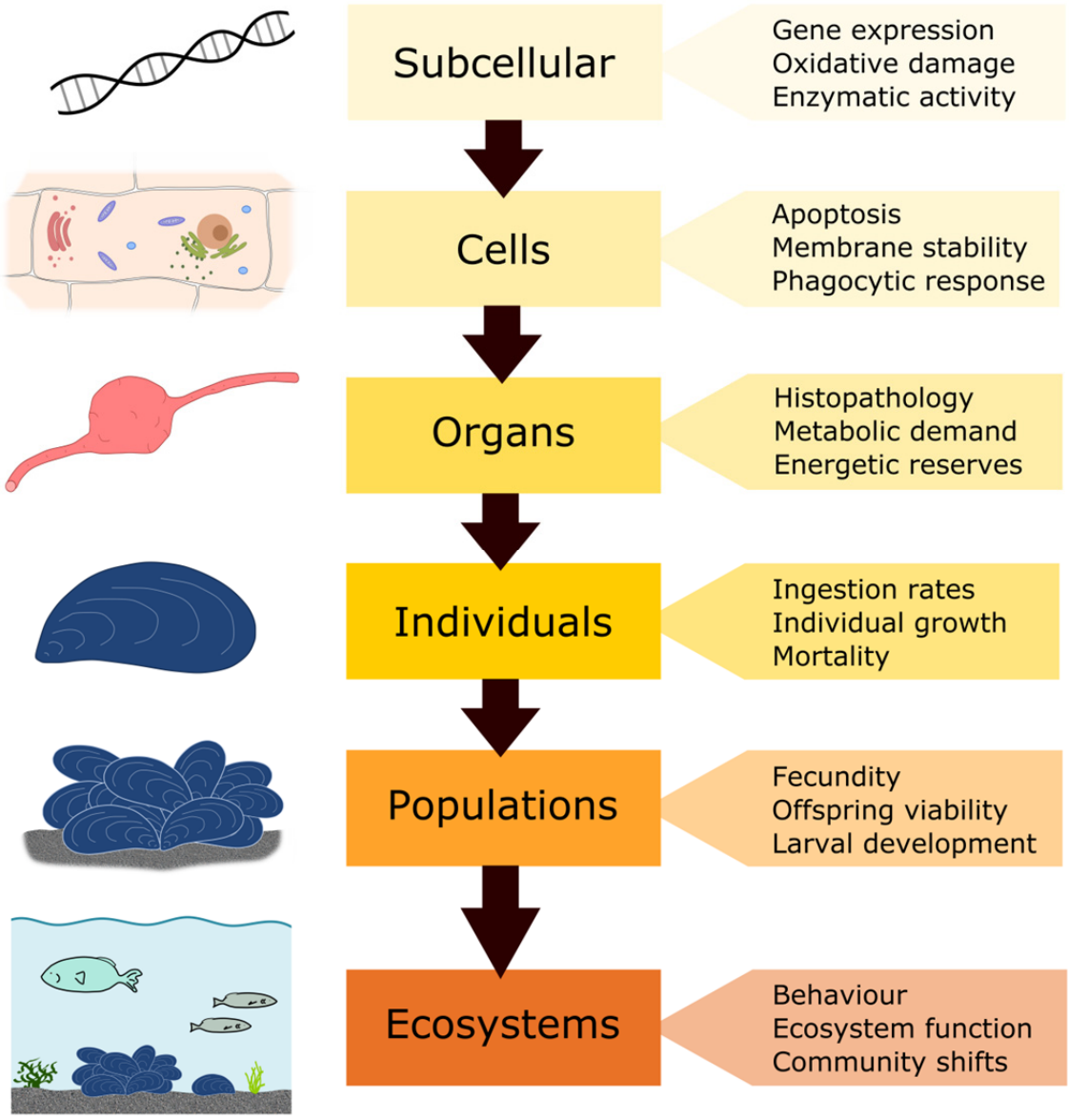

Figure 5. Microplastics can affect a number of biological endpoints (right) on different levels of biological organisation (left). Effects may cascade from one level to the next. Figure modified from Galloway et al. (2017).

3.4.1 From cells to organs

On a subcellular level, exposure to microplastics has been found to modulate gene expression in several species, in particular genes related to cellular

stress response and immune functions (Détrée and Gallardo-Escárate, 2018; Liu et al., 2019; Pinsino et al., 2017). Also, the transcription of genes involved in energy metabolism and development can be affected by the exposure to plastic particles, as observed in the Pacific oyster *Crassostrea gigas* (Sussarellu et al., 2016). In accordance with these observations, changes in enzymatic activity and cellular stress responses have been found (Della Torre et al., 2014; Gambardella et al., 2017). For instance, in *M. galloprovincialis* few hours of exposure to 1 mg L^{-1} of 50 nm amino-modified PS beads led to an increased production of reactive oxygen species, along with production of nitric oxide, and induced phagocytosis (Canesi et al., 2015). Oxidative stress was also observed in *M. edulis* after 96h exposure to 100 or 1,000 PE particles mL^{-1}, as indicated by fluctuations in the activity of antioxidant enzymes (Magara et al., 2018). Furthermore, blue mussels exhibited strong inflammatory responses and tissue alterations after few hours of exposure to 80 µm HDPE particles, which emerged at very high concentrations of 2.5 g L^{-1} (von Moos et al., 2012). However, structural changes in the tissue were also observed in Mediterranean mussels at a lower concentration (0.01 g L^{-1}) and longer exposure time (21 days) with PE particles (Bråte et al., 2018).

3.4.2 Effects on individuals

Exposure to microplastics for up to 4 weeks has been found to decrease energy reserves in the sediment-dwelling bivalves *Ennucula tenuis* and *Abra nitida* (Bour et al., 2018), and the lugworm *Arenicola marina* (Wright et al., 2013a). This has been related to changes in feeding activity – a biological endpoint that has commonly been shown to be affected by the presence of plastic particles – for instance in the copepod *Calanus helgolandicus* (Cole et al., 2015), the Asian green mussel *Perna viridis* (Rist et al., 2016), the langoustine *Nephrops norvegicus* (Welden and Cowie, 2016b) and the freshwater cnidarian *Hydra attenuata* (Murphy and Quinn, 2018). The feeding rate of *D. magna* was decreased by 21% when animals were exposed to 100 nm PS beads at 1 mg L^{-1}. This was possibly caused by the particles interacting with the filter setae and/or the gut wall, thereby disturbing the feeding process (Rist et al., 2017 - Paper I). Particles with a size of 2 µm did not cause an effect in the same study. Contrarily, Ogonowski et al. (2016) found a reduction of daphnid feeding rates by 29% caused by 4.1 µm plastic beads at a concentration of $2.25 \cdot 10^5$ beads mL^{-1}. Despite similarities between the studies concerning particle size (2 vs. 4.1 µm) and concentration ($1.4 \cdot 10^5$ vs. $2.25 \cdot 10^5$ beads mL^{-1}), the deviating outcomes may be related to differences in the particle composition (PS vs. unknown plastic) or experimental handling. Further

physiological endpoints that have been shown to be affected following exposure to microplastics are respiration (Rist et al., 2016; Watts et al., 2016) as well as biomass production and growth (Besseling et al., 2014; Ogonowski et al., 2016; Welden and Cowie, 2016b).

While invertebrates are often able to compensate or recover from the effects of a stressor, a severe or chronic stress may decrease the chance of survival. Several studies have reported mortality caused by the exposure to microplastics, for instance in *P. viridis* (Rist et al., 2016), *P. pugio* (Gray and Weinstein, 2017), *H. azteca* (Au et al., 2015) and *D. magna* (Ogonowski et al., 2016). This was, however, mostly observed at very high particle concentrations. The lowest exposure level that resulted in a substantial increase of mortality was 50 PP microfibres mL^{-1}, at which 55% of exposed *P. pugio* died (Gray and Weinstein, 2017). Lower levels of mortality were found for PP fragments as well as PE and PS beads at the same concentration, further indicating that particle shape is an important factor for toxicity. Accordingly, in *H. azteca,* exposure to PP fibres resulted in a stronger effect on mortality compared to PE fragments (Au et al., 2015). It should, however, be noted that also an influence of particle composition cannot be excluded, when the particles differ with regards to the polymer and possibly associated chemicals.

3.4.3 Effects on a population level

Potential effects of microplastics on reproduction and development are of particular interest since impacts on this level may affect whole populations, and early development stages are especially sensitive towards environmental stressors (His et al., 1999; Widdows, 1991). When blue mussel larvae were exposed to 100 nm and 2 µm PS beads at concentrations between 0.45 and 287 µg L^{-1} for up to 15 days, developmental abnormalities were observed (Rist et al., 2018a - Paper V). This was more pronounced for the smaller particles, higher concentrations and longer exposure times (Fig. 6). These results could indicate that particles in the nanometre size are more hazardous, possibly related to their higher chance of interacting with, and crossing, cellular membranes (da Costa et al., 2016). Also in other studies differences in responses to microplastics depended on particle size. However, both smaller (Cole et al., 2013) and bigger (Bour et al., 2018) particles have been found to induce stronger effects. Particle size is therefore an important factor and may influence effects of microplastics differently depending on the biological endpoint. The stronger effect on *M. edulis* larval development caused by 100 nm in comparison to 2 µm particles (Rist et al., 2018a - Paper V) may also be a matter of different particle numbers for both sizes at the same mass concen-

tration. Although mass concentrations are often applied in effect studies, particle numbers are important to consider since it is the individual entities that an organism interacts with (Connors et al., 2017).

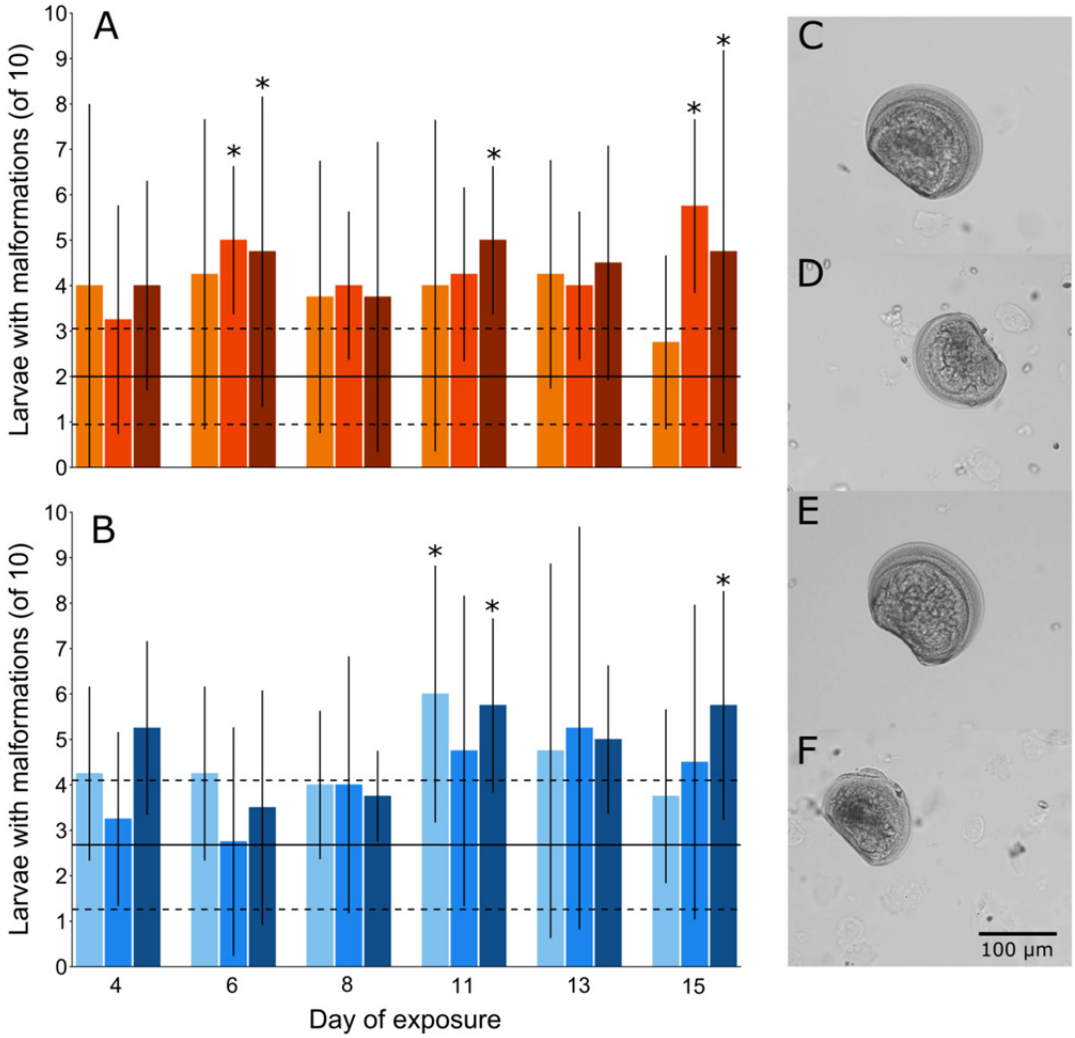

Figure 6. Number of blue mussel (*Mytilus edulis*) larvae (counted in a subset of 10 from every replicate, n=4) showing malformations during 15 days exposure to 100 nm (A) or 2 µm (B) polystyrene beads at a low (0.45 µg L^{-1}, lightest colour), intermediate (28.7 µg L^{-1}, intermediate colour) and high (287 µg L^{-1}, darkest colour) concentration. The horizontal line illustrates the mean of the pooled controls with the standard deviation (dashed lines, n=24). Significant differences ($p<0.05$) between a treatment group and the control are indicated by an asterisk (*). C shows a normally developed mussel larva at day 13. Commonly observed malformations were convex hinge (D), cupped (E) and protruding mantle (F). Figure modified from Rist et al. (2018a - Paper V).

Impairment of normal development was also found in larvae of *M. galloprovincialis* at similar concentrations of plastic particles (median effect concentration (EC_{50}) of 142 µg L^{-1}) as in the study with *M. edulis* larvae, but after

only 48h and with 50 nm amino-modified PS beads (Balbi et al., 2017). The same particles resulted in an EC_{50} value of 150 µg L^{-1} (36h) for abnormal development in *C. gigas* larvae, whereas unmodified 50 nm PS beads only led to effects at much higher concentrations of 10 to 25 mg L^{-1} (Tallec et al., 2018). This emphasises that surface chemistry not only plays a role for particle uptake and translocation, but also for effects, with cationic particles most likely being more hazardous to organisms. The fact that Rist et al. (2018a - Paper V) still observed effects at relatively low concentrations with plain PS particles may be attributed to the long exposure time – a decisive factor for the development of effects.

Many factors have been identified that can influence potential effects of microplastics on organisms, including particle size (e.g. Cole et al., 2013; Rist et al., 2017 - Paper I), shape (e.g. Au et al., 2015; Gray and Weinstein, 2017), surface chemistry (e.g. Tallec et al., 2018), concentration (e.g. Balbi et al., 2017) and exposure duration (e.g., Rist et al., 2018a - Paper V). This might explain why, even at very similar experimental conditions with the same species, results can greatly vary, such as in the case of reproduction in *D. magna*. While exposure to 0.1, 0.5 and 1 mg L^{-1} of 2 µm PS beads did not impair reproductive success of the animals in a study by Rist et al. (2017 - Paper I), Martins et al. (2018) found 0.1 mg L^{-1} of 1 to 5 µm beads to significantly reduce the number of offspring, even leading to a complete extinction of the plastics-exposed population in the F_1 generation. This makes it difficult to draw general conclusions from individual studies and could speak for the implementation of standardised testing guidelines that include, for instance, standard particles and defined exposure characterisation (see Chapter 5).

4 Interactions between plastics and chemicals

4.1 Leaching and sorption of chemicals

Interactions between plastics and chemicals are important to consider in the context of potential effects of microplastics on organisms. Two main processes can be distinguished: 1) Release or leaching of chemicals from the plastic, and 2) sorption of chemicals from the environment to the plastic (Fig. 7).

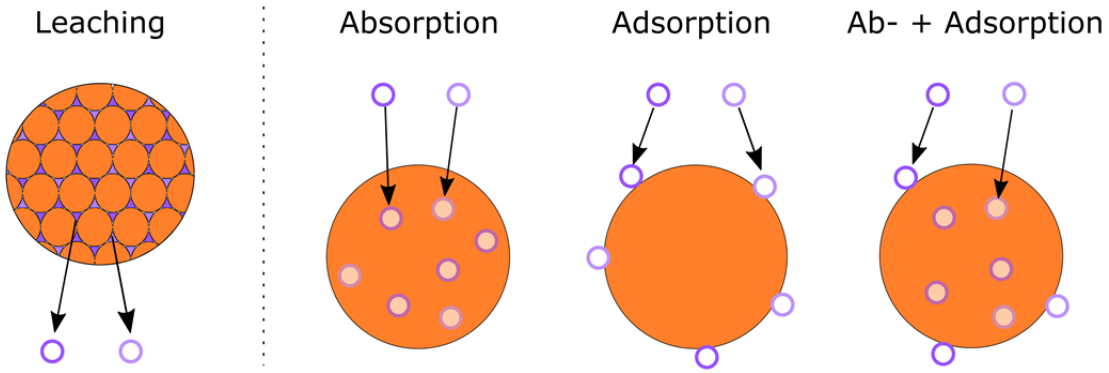

Figure 7. Leaching describes the release of chemicals (purple-white), which were part of the plastic material (orange). Sorption is the process of chemicals sorbing to plastics upon contact in the environment. This can occur as absorption, adsorption or a combination of both.

Plastic materials are primarily composed of certain polymers. Additionally, they can contain a multitude of chemicals from the production, which can potentially leach out of the material (Fig. 7) and become available to organisms (Hahladakis et al., 2018). This includes additives that were intentionally incorporated to give the material certain properties, but also monomers and oligomers from incomplete polymerisation, catalysts and solvents from the production process, impurities and break-down products (Muncke, 2009). Leached chemicals can be transported to organisms and cause effects (e.g. Li et al., 2016; Nobre et al., 2015). This has, for instance, been demonstrated in the brown mussel *Perna perna*, in which leachates of PP pellets caused abnormal embryonic development (Gandara e Silva et al., 2016). Mostly, complete information on the composition of a plastic material is not available,

even for materials of known origin (Groh et al., 2019). This hampers the evaluation of the potential chemical toxicity that a certain material might exhibit.

Additionally to plastic-associated chemicals stemming from production, plastic particles come into contact with HOCs at different stages: before, during and after their release to the environment (Rist and Hartmann, 2018 - Paper III). Because of their hydrophobicity, HOCs sorb to the nonpolar plastic material. It has been found that they can accumulate on microplastics, exhibiting concentrations that are up to 1 million times higher than in the surrounding seawater (Mato et al., 2001; Rios et al., 2007; Teuten et al., 2007). Especially small plastic particles have a comparatively big sorption capacity due to their high surface-to-volume ratio. The degree of sorption depends on the properties of the plastic particle, the HOC and the surrounding medium (Rist and Hartmann, 2018 - Paper III). The governing process for the distribution of HOCs between the water phase and particulate matter is molecular diffusion and can be described by equilibrium partitioning (Hartmann et al., 2017 - Paper VII).

Sorption can take place as absorption, adsorption or a combination of both (Fig. 7). In the process of absorption, molecules partition into the sorbing matrix (i.e. the plastic particle). The molecules are dissolved and only bound by relatively weak van-der-Waals forces. Absorption is largely driven by the hydrophobicity of the HOC. During adsorption molecules bind to the surface of the plastic particle which involves a variety of interaction forces, such as van-der-Waals, ionic, steric, and covalent bonds (Hartmann et al., 2017 - Paper VII). At low concentrations of HOCs, adsorption is the dominant process due to strong interaction forces on the particle's surface. However, at high concentrations, absorption becomes dominant since the larger volume can accommodate more molecules (Cornelissen et al., 2005; Luthy et al., 1997).

Within the polymer, the HOC transport depends on several factors, including the segmental mobility of the polymer chains and the free volume in the polymer (Rusina et al., 2010). This is determined by the crystallinity and the degree of cross linking. Plastic polymers contain crystalline and amorphous regions. HOCs generally sorb in amorphous regions, which have a less fixed and ordered structure (Teuten et al., 2009). Depending on the glass transition temperature, the amorphous region can be classified as glassy or rubbery, whereby glassy polymers (e.g. PS and PVC) are more cross-linked than rub-

bery polymers (e.g. PE and PP). Accordingly, glassy polymers have a lower diffusivity, and adsorption was found to dominate for these, while absorption dominated in rubbery polymers (Hüffer and Hofmann, 2016). Particle size and shape is also of importance since it determines the diffusional length scale and the surface-to-volume ratio. The surface polarity plays a role as well and is influenced by chemical modifications and coatings, but also by transformation processes in the environment (Teuten et al., 2009). For instance, biofilms that are formed during biofouling can constitute additional sorbents as well as barriers for diffusive transport (Endo et al., 2005; Rummel et al., 2017).

With regards to the HOCs, important properties are molecular weight, molar volume and hydrophobicity (Hartmann et al., 2017 - Paper VII). The lower the molecular weight and molar volume and the higher the hydrophobicity, the faster is the diffusive mass transfer and the establishment of an equilibrium (Müller et al., 2001; Pascall et al., 2005; Rochman et al., 2013b). The degree of desorption of a HOC from a plastic particle depends on many factors but is generally higher at low partitioning ratios and low binding strength. Therefore, absorbed HOCs are released more readily than strongly bound adsorbed HOCs (Cornelissen et al., 2005; Mayer et al., 2011). As mentioned above, the interaction between microplastics and HOCs is also influenced by the conditions in the surrounding medium, including temperature, pH and ionic strength (Hartmann et al., 2017 - Paper VII). For instance, partitioning of phenanthrene and polychlorinated biphenyls (PCBs) to plastic particles was found to increase at higher salinity (Karapanagioti and Klontza, 2008; Velzeboer et al., 2014). Furthermore, HOC desorption from plastics increases at elevated temperature and lower pH (Bakir et al., 2014).

4.2 Plastics as vectors for chemicals

The sorption to plastics can enhance the mobility of HOCs in the aquatic environment. As a result, the global distribution and bioavailability of these chemicals may be increased, including substances with known hazard to aquatic animals (Rochman, 2015). It has also been demonstrated that pollutants can move from plastic particles into animal tissues upon particle ingestion (Besseling et al., 2013; Chua et al., 2014), with subsequent adverse effects on the organisms (Browne et al., 2013; Paul-Pont et al., 2016). Altogether, these aspects have led to the assumption of microplastics being a significant vector for chemicals – also referred to as the Trojan horse effect. As

illustrated in Fig. 8, several types of vector effects can be distinguished (Syberg et al., 2015; Rist and Hartmann, 2018 - Paper III): 1) The environmental vector effect describes the particle-mediated transport of a pollutant through the environment. 2) Co-transport into organisms is referred to as the organismal vector effect, while 3) the cellular vector effect is the co-transport into cells. However, the interaction between microplastics, pollutants and organisms/cells can occur in a variety of ways. Besides a direct co-transport, the interaction can take place via desorption and subsequent transfer of the pollutant (Fig. 8, 2b). Furthermore, sorption to plastic particles can decrease the availability of a pollutant to an organism and plastic particles can even lower the concentration of pollutants in organisms (Fig. 8, 2d) by sorbing pollutants if they are present at higher concentration in the animal tissue than on the plastic (Ziccardi et al., 2016).

The process of chemical transfer depends on the specific chemical pollutant, the properties of the plastic particle and the conditions of the surrounding medium, including the organism (Rist and Hartmann, 2018 - Paper III). It has for instance been found that gut surfactants can greatly enhance the desorption of chemicals from microplastics, especially in warm blooded animals (Bakir et al., 2014). An even higher mass transfer can be expected via direct contact exposure (Fig. 8, 2c) when plastic particles adhere to body structures of an animal, either externally (e.g. on skin or exoskeleton) or internally after uptake (e.g. on gut or gill walls) (Hartmann et al., 2017 - Paper VII). Different processes of diffusive mass transfer of polycyclic aromatic hydrocarbons (PAHs) were investigated with a PAH-loaded and a clean silicone disk. It was found that transfer increased when the disks were separated by gut fluid of a sediment dwelling worm in comparison to water, but was highest when the disks were in direct physical contact (Mayer et al., 2007). Accordingly, Hartmann et al. (2017 - Paper VII) suggested that HOC transfer from microplastics to organisms happens faster through gut fluid than water and fastest upon direct contact of the particles with external or internal tissues – a mechanism that has so far been overlooked.

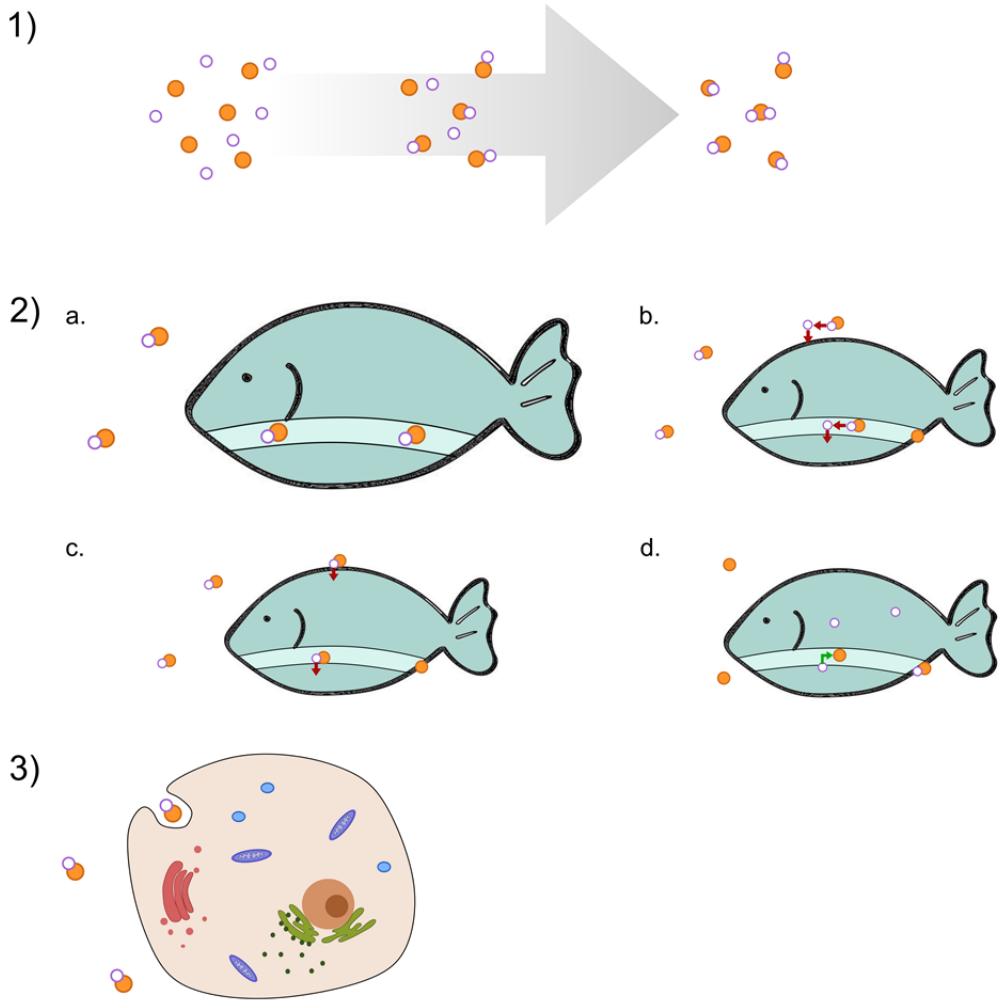

Figure 8. Microplastics (orange spheres) can act as vectors for organic pollutants (purple-white spheres) in different ways: 1) by co-transport through the environment (environmental vector effect), 2a) by co-transport to organisms (organismal vector effect), and 3) by co-transport into cells (cellular vector effect). During transfer to an organism, the pollutant can either desorb from the plastic and subsequently sorb to the animal tissue (2b) or it can be mediated by direct contact exposure (2c). Microplastics may also clean an organism by sorbing a pollutant from the tissue (2d). Figure modified from Rist and Hartmann (2018 - Paper III) and Hartmann et al. (2017 - Paper VII).

It is important to note that other matrices in the aquatic environment also sorb pollutants and transfer these to organisms, such as natural inorganic particles, organic matter and natural prey. In an experiment with *A. marina*, Browne et al. (2013) found sand to transport more pollutants to the animals than PVC particles. A similar observation was made for algae that were provided as food when *Mytilus* spp. was exposed to PS beads and fluoranthene (Paul-Pont et al., 2016). This was the case even though the PS beads had a higher sorption capacity for fluoranthene than the algae but the latter was present in

larger mass. When reviewing the literature about the role of microplastics as a vector for HOCs, Burns and Boxall (2018) concluded that results of available studies either do not support a significant vector effect, as for example demonstrated by other matrices transporting more HOCs to organisms, or are inconclusive. The latter was mainly related to unrealistic test gradients, for instance when using highly HOC-loaded particles in clean water, and study designs that are insufficient in testing the vector effect. Ziccardi et al. (2016) came to a similar conclusion and pointed out that most studies also reported effects of microplastics alone. To elucidate the role of microplastics as vector for HOCs to aquatic organisms, studies using more realistic designs are therefore urgently needed.

On a global scale, the role of plastics as a carrier for HOCs was estimated to be negligible in comparison to natural matrices in the marine environment, including water, dissolved organic carbon, colloids, detritus, black carbon, plankton and bacteria (Bakir et al., 2016; Koelmans et al., 2016; Ziccardi et al., 2016). This is mainly related to the enormous differences in mass, resulting in the estimation that plastics only hold 0.0002% of HOCs in the oceans (Koelmans et al., 2016). However, this is a global average and since plastics are not distributed evenly in the environment, different conditions will apply depending on the location. On a small scale, plastics may constitute a significant matrix, for instance at hotspots of plastic pollution, such as ocean gyres (Cozar et al., 2014), the vicinity of plastic production plants (Karlsson et al., 2018; Noren, 2007) and coastal sediments (Carson et al., 2011; Lee et al., 2015). Recently, it has for instance been found that the dry mass of plastics (>0.5 mm) was on average 180 time higher than of biota in surface waters of the North Pacific accumulation zone (Chen et al., 2018). A majority (84%) of the plastics had chemicals exceeding threshold effect concentrations, leading the authors to the conclusion that the plastics may be significant in transferring chemicals to certain marine organisms. Another aspect to consider is that the abundance of microplastics is most likely underestimated since it is currently not possible to reliably quantify the smallest size fraction of a few micrometres and below. This could be of importance as it has been found that particle numbers increase for smaller sizes (Song et al., 2015). Besides, smaller particles exhibit a higher sorption capacity due to the higher surface-to-volume ratio. It is also expected that plastic inputs into the aquatic environment will rise further in the future (Jambeck et al., 2015), potentially increasing the role of plastics as a sorbent for HOCs.

5 Recommendations for an enhanced understanding of the exposure system

5.1 Control in exposure tests

Two main approaches can be followed when conducting experimental exposure studies with microplastics: 1) Using an exposure system that simulates the natural environment as closely as possible (e.g. mesocosms), or 2) designing a system that is as controlled as possible. There is a trade-off between environmental realism and control (Wickson et al., 2014), and it should carefully be considered which approach is more suitable for the aim of a study since both have advantages and limitations. Environmentally realistic studies provide more case- and site-specific information, while standardised, controlled tests using a simplified test system may give a deeper mechanistic insight and causal explanations. Both approaches aim at answering different questions and should therefore be seen as complementary to each other (Rist and Hartmann, 2018 - Paper III).

Microplastic effect studies greatly differ in the used conditions and parameters with regards to particle types (variations in size, shape, chemical composition, charge), particle concentrations, exposure durations, test organisms and biological endpoints. Information on the used systems is not always fully provided in studies (Connors et al., 2017), which makes it difficult to compare and reproduce results. Therefore, it could be beneficial to use more standardised test systems as it has been discussed and implemented in the work with ENMs (Rist and Hartmann, 2018 - Paper III). Several studies have used standard ecotoxicity tests in the work with microplastics, for instance the acute (48h) immobilisation test with *D. magna* (Booth et al., 2016; Casado et al., 2013; Rehse et al., 2016). It is, however, questionable how suitable these tests are when applied to microplastics since they have been developed for testing soluble chemicals. Microplastics are inherently different and more complex due to a variety of influencing factors like, particle shape, size and composition, and the dynamic nature of particles in solution.

Similar problems have been recognised in the work with ENMs, leading to the development of standard procedures for particle and exposure characterisation (Hjorth et al., 2017). It has been suggested that the experiences made in the field of ENMs should be used in the work with microplastics since many of the challenges are comparable (Connors et al., 2017; Rist and

Hartmann, 2018 - Paper III). A thorough characterisation provides important information for understanding particle behaviour in the test system as well as the interaction with the test organism, and helps preventing test artefacts (Petersen et al., 2014). It should include analyses of particle shape, size, composition, density, surface chemistry and charge (Fig. 9, A). Particle suspensions are not necessarily stable. Therefore, the aggregation/agglomeration behaviour of the particles as well as the sedimentation needs to be assessed in order to evaluate the actual exposure conditions for the organism (Fig. 9, C and D). To achieve suspensions which are as stable and as reproducible as possible, appropriate methods for dispersing particles should be developed and reported (Fig. 9, B).

It is also important to consider that particle properties can change during an experiment. On a short time scale, particles may interact with natural organic macromolecules (NOM) in the water, forming a layer of NOM on the particle surface, which is referred to as the ecocorona (Galloway et al., 2017). For instance, proteins secreted by *D. magna* were found to attach to the surface of PS beads, which subsequently enhanced their ingestion by the daphnids and resulted in higher toxicity than clean beads (Nasser and Lynch, 2016). On a longer time scale, oxidative degradation processes can affect particles by changing the surface chemistry, polarity and density (Jahnke et al., 2017). Thus, it is recommendable to examine possible transformation processes also during the course of an experiment (Fig. 9, E).

The work with ENMs has greatly profited from the use of reference materials (Connors et al., 2017; Rist and Hartmann, 2018 - Paper III). These are well characterised and standardised particles made of industrially and environmentally relevant materials of different size and composition. They have proven useful for the validation of analytical methods as well as for inter-laboratory and inter-species comparisons. Reference materials could therefore be used to increase the comparability and reproducibility of microplastic effect studies (Fig. 9, F).

Often it is not clear where particles end up in an exposure system. However, it has been demonstrated that particle fate in the system can be elucidated by making a mass balance of all particles (Rist et al., 2018c - Paper IV). This was achieved in an exposure study with *M. edulis* by counting particles in different compartments: in mussels after exposure, in the exposure water, in mussels after a subsequent depuration period and in the water after depuration. The high recovery rate showed that it is possible to follow the fate of

close to all particles at low, environmentally realistic particle numbers. This can help to understand the interaction of particles and test organisms.

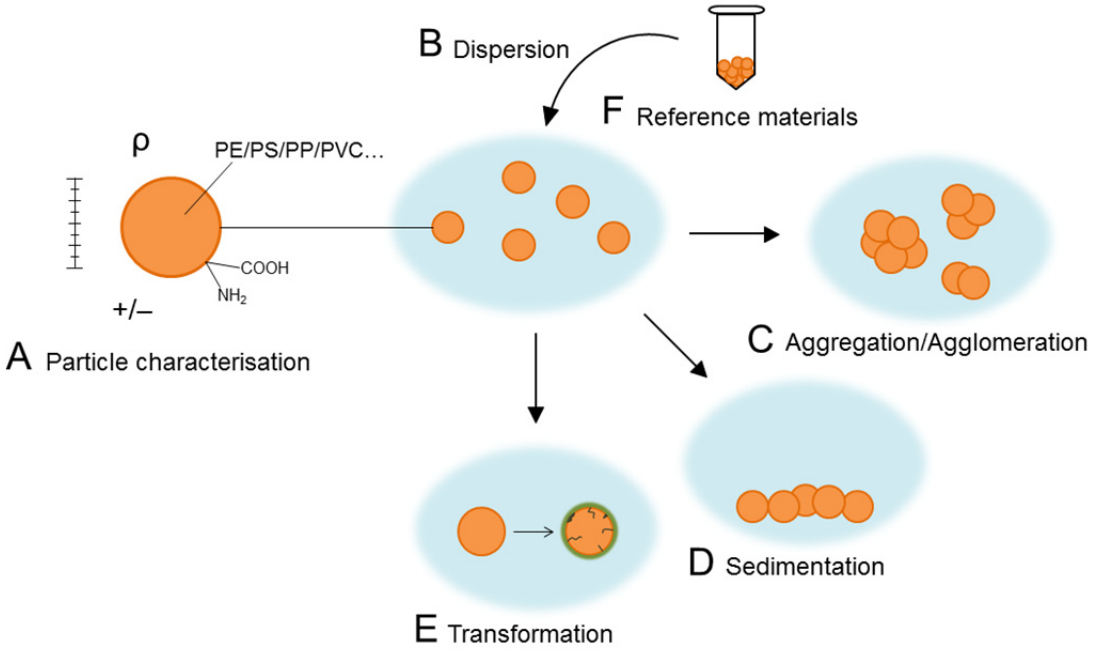

Figure 9. A detailed particle and exposure characterisation is recommended for microplastic exposure tests. Particle characterisation (A) should include analyses of particle shape, size, composition, density, charge and surface chemistry. Reliable methods for particle dispersion (B) are needed and particle aggregation/agglomeration (C), sedimentation (D) and transformation (E) should be measured. In some cases the use of reference materials (F) can be recommendable.

It is a matter of debate whether highly controlled or even standardised tests should be employed in the work with microplastics, similar to the field of ENMs. As discussed, this could bring several benefits. However, it requires adaption of existing or development of new tests, which are more applicable to particles. It is especially important to identify sensitive biological endpoints, possibly moving away from current standard test organisms. In this respect, it has been suggested that cellular responses may be more sensitive than organismal effects, such as immobilisation, since many changes must have taken place on a cellular level before the latter occurs (Rist and Hartmann, 2018 - Paper III). However, this could also make analyses more complex, requiring specialised techniques and knowledge. On a physiological level, animal feeding may be a promising endpoint since this has been identified as one of the most consistent effects of microplastic exposure across different aquatic species (Foley et al., 2018). To achieve higher sensitivity, also

at lower particle concentrations, chronic instead of acute studies should be favoured. There are still many uncertainties related to the mechanisms by which microplastics affect organisms on different levels. Thus, in order to choose sensitive species and biological endpoints for standardised tests with microplastics, more research is needed that specifically addresses this.

5.2 Particle versus chemical effects

The majority of microplastic effect studies focus on the physical nature of the particle, indicating an underlying notion that plastic particles are inert. On the other hand, a number of studies have investigated the effects of chemicals that are co-transported to organisms by microplastics, as discussed in Chapter 4.2. However, plastic materials can also exhibit a chemical effect without the sorption of pollutants since the material itself mostly contains a variety of different chemicals, which can leach from the particles and in this way become available to organisms. It has also been demonstrated that leachates of microplastics can adversely affect aquatic invertebrates, for instance larvae of the barnacle *Amphibalanus amphitrite* (Li et al., 2016), the sea urchin *Paracentrotus lividus* (Martínez-Gómez et al., 2017) and *P. perna* (Gandara e Silva et al., 2016). In most studies though, it is not possible to distinguish physical and chemical effects of the used particles since they occur simultaneously. Therefore, it is recommendable to include chemical leaching controls in exposure studies that aim at investigating the particle-related effects of microplastics (Rist and Hartmann, 2018 - Paper III). A similar challenge has widely been recognised in the work with ENMs – dealing with the release of metal ions from metal nanoparticles – which has led to the ongoing development of test guidelines on particle dissolution (Baun et al., 2017).

In addition to chemicals potentially leaching from the particles, there may be chemicals added to the final product when particles are purchased. Especially microbeads, which are most commonly used in microplastic effect studies, are often supplied in solutions containing surfactants to ensure particle dispersion as well as other chemicals such as preservatives. It has already been suggested to include surfactant controls (Connors et al., 2017) and this was done in some studies (Choi et al., 2018; Khan et al., 2015). However, such controls are not sufficient to test the total chemical toxicity that particles may exhibit and therefore a chemical control that also tests for leaching should be favoured. Such a control could be implemented by keeping the tested particles in the water that is used for the exposure test under comparable condi-

tions (same particle concentration, temperature, light, duration etc. as in the test) and filtering the particles out before the start of the test. Another possibility is to use an exposure tank in which the particles are physically separated from the organisms, for example by a mesh, while the water and potential chemicals can move freely between both compartments.

5.3 Increasing the environmental realism

In recent years, it has increasingly been criticised that a majority of microplastic effect studies lack environmental realism in their design, questioning whether effects observed in laboratory studies are translatable to the field (Burns and Boxall, 2018; Connors et al., 2017; Lenz et al., 2016; Ogonowski et al., 2018; Phuong et al., 2016). Some major points of this critique are the used microplastic concentrations, particle types and the absence of comparisons with natural particles (Fig. 10).

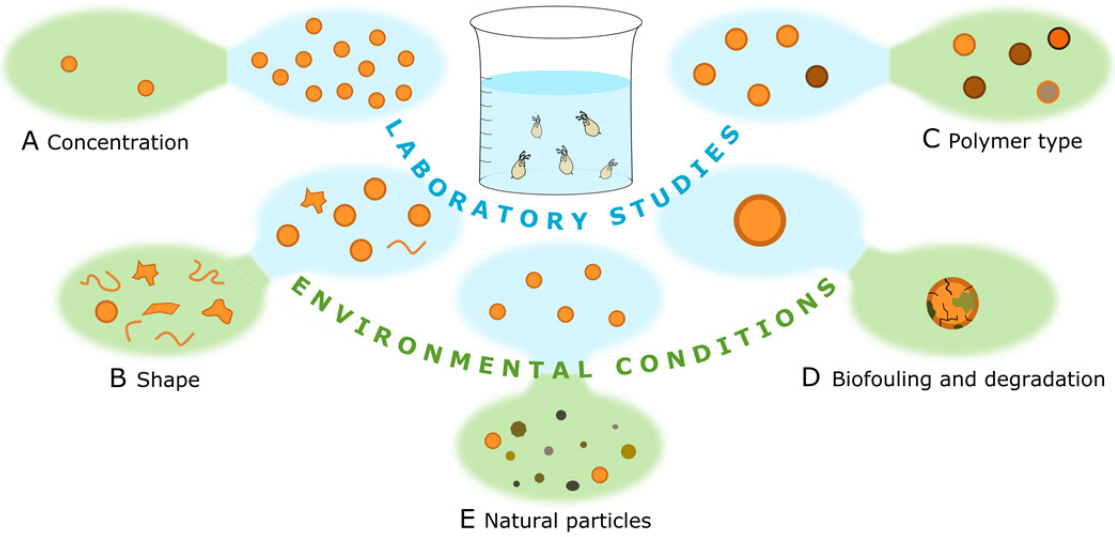

Figure 10. The conditions used in a majority of laboratory microplastic exposure studies (blue) differ greatly from those in the aquatic environment (green). This mainly refers to particle concentrations (A), shapes (B), polymer types (C), biofouling and degradation (D), and the presence of natural particles (E).

Particle concentrations (Fig. 10, A) that have commonly been used in exposure studies are 2 to 9 orders of magnitude higher than levels that have been reported from the aquatic environment (Connors et al., 2017; Lenz et al., 2016). The use of high concentrations can be beneficial for a proof of concept

or mechanistic studies, but with regards to effects on organisms, results need to be interpreted with care. If the concentrations at which effects were observed are not put into an environmental context, this may result in misinterpretation and overstatements of risks posed by microplastics (Lenz et al., 2016). However, it is also important to acknowledge that microplastic concentrations in the field come with uncertainties. The detected particle numbers can greatly be influenced by the used methods, questioning the comparability and reliability of results (Burns and Boxall, 2018). When comparing a common manual with a new automated approach to analyse microplastics in an environmental sample with micro-Fourier transform infrared microscopy, Primpke et al. (2017) found almost 7 times more particles with the automated approach. The main cause for this difference was that particles <30 µm were often missed during manual analysis. This indicates that, despite much technical advancement in the last years, microplastic numbers are likely underestimated with currently used methods. Furthermore, technical limitations do not allow reliable quantification of particles <10 µm yet. It is thus possible that microplastic abundance in environmental samples is systematically underestimated to date. With regards to microplastic exposure studies, it is therefore relevant to test a range of different concentrations, but this should include lower levels as well. In order to increase the comparability between studies, particle concentrations should be given as both masses and numbers. It has been argued that the use of particle numbers is more relevant (Ogonowski et al., 2018), but since both are commonly used in exposure studies, all necessary information to convert one to the other should be given (Connors et al., 2017).

An aspect that also makes it difficult to project results from laboratory studies to the field is the widespread use of microplastic beads (Fig. 10, B). In contrast, fragments and fibres are the most common particle shapes found in environmental samples (Burns and Boxall, 2018). Moreover, fibres were the dominant shape that zooplankton had ingested in the Northeast Pacific (Desforges et al., 2014) and South China Sea (Sun et al., 2017). Since particle shape can influence the interaction between organisms and microplastics (see Chapter 3), fragments and fibres should be used more frequently in effect studies. Currently, this is hampered by the fact that, in contrast to beads, standardised fragments and fibres are not readily available for purchase and difficult to prepare in small sizes. Some method development has been made in recent years. For instance, Cole (2016) reported a method for preparing microfibres in a length of 40 to 100 µm using a cryotome. Fragments in wide

size ranges have been obtained from different companies (Gray and Weinstein, 2017; Rist et al., 2016) as well as produced by milling (Eitzen et al., 2018; Hämer et al., 2014; Kühn et al., 2018). Subsequent sieving allowed fractionation of different sizes. However, several methodological challenges remain, such as dispersing fragments and applying the method to a wider range of polymers (Eitzen et al., 2018). More method development for creating standardized particles, or collaborations with industries where this is frequently done, is therefore urgently needed. In the best case, different particle shapes should be available for a variety of polymers since there is also a mismatch between laboratory studies and the environment in this respect (Fig. 10, C). While the former mostly use PS, the variety of polymer types is much greater in environmental samples, with PE, PET, PA and PP being some of the most commonly found polymers (Burns and Boxall, 2018; Phuong et al., 2016). In order to reflect the diversity of microplastics in the field, it is also a promising approach to conduct effect studies with particle mixtures (Imhof et al., 2017).

Plastic particles in the environment are subjected to degradation processes and quickly interact with organic matter and microorganisms, resulting in biofouling. The latter has been shown to affect particle-organism interaction (Bråte et al., 2018). It is therefore recommendable to include biofouling in effect studies to increase their environmental relevance (Fig. 10, D). Besides the use of new plastic materials, it is also possible to prepare test particles from plastic debris collected in the environment. Using cryo-milling, Kühn et al. (2018) recently made a mixture of microplastics that was representative of the macroplastics collected on a beach on Texel (Netherlands). This provides a valuable method for obtaining particles that can be considered almost environmentally realistic. However, this is very site-specific and the unknown history and chemical composition of the collected plastics requires an array of chemical and physical characterisation methods (Kühn et al., 2018).

A major issue of microplastic effect studies is that in most cases it remains unclear whether observed effects are microplastic-specific or suspended-particle-effects (Fig. 10, E), meaning that similar results would be obtained with a natural particle of comparable size and behaviour (Connors et al., 2017; Ogonowski et al., 2018). Aquatic invertebrates live in an environment that naturally contains suspended solids and many natural particles overlap with microplastics in size, including sand, clay and silt (Connors et al., 2017). This is not considered in the majority of effect studies. Therefore, including a control with a natural particle of defined size and properties is highly recom-

mended. The main challenge is to find a reference particle that is as similar to the tested microplastics as possible. It is not likely to find an ideal natural reference – some properties will always differ from the tested plastic particle – but a range of natural particles are available, including clay, silica, glass beads and cellulose, from which the most comparable can be chosen (Ogonowski et al., 2018). Some studies, which included a natural particle control, reported effects that only emerged in the plastic treatments, such as weight decrease in the amphipod *Gammarus fossarum* caused by poly(methyl methacrylate) (PMMA) and polyhydroxybutyrate (PHB), but not silica (Straub et al., 2017), or lower feeding rates of *D. magna* under the exposure to microplastic beads and fragments, but not kaolin (Ogonowski et al., 2016). While these results indicate microplastic-specific effects, it remains unclear which particle properties the differences in response were based on. This requires studies that specifically address this question and it has been emphasised that not enough information is available yet to conclude that effects of microplastics consistently differ from those of natural particles (Ogonowski et al., 2018).

6 (Micro-)plastic pollution in a broader perspective

6.1 Human exposure to microplastics and associated chemicals

While the focus of this thesis has so far mainly been on microplastic interactions with and impacts on aquatic invertebrates – a major area within this research field – it is highly relevant to discuss human exposure to microplastics and potential effects. Even though research on microplastics in relation to humans is very limited to date, the topic has received much attention in the scientific and public debate in recent years. Widespread observations of microplastic ingestion in different aquatic organisms, including species for human consumption, as well as reports on trophic transfer of microplastics have led to the assumption that microplastics will ultimately end in food for human consumption. This has triggered a discussion on adverse effects of microplastics on human health (Rochman et al., 2015b; Vethaak and Leslie, 2016; Rist et al., 2018b - Paper II). Besides studies on the presence of plastic particles in seafood (Rochman et al., 2015b; Van Cauwenberghe and Janssen, 2014), this discussion has been fuelled by reports of microplastics in other food products and beverages, such as table salt (Karami et al., 2017; Yang et al., 2015), honey (Liebezeit and Liebezeit, 2013), drinking water (Kosuth et al., 2018; Mason et al., 2018) and beer (Kosuth et al., 2018; Liebezeit and Liebezeit, 2014). As a consequence, concerns have been raised about safe consumption of these products. There is, however, a big discrepancy between the extent of the discussion on human health effects of microplastics and actual scientific evidence. Studies have merely reported the potential presence of microplastics in a variety of products and were not designed to evaluate potential impacts on human health (Rist et al., 2018b - Paper II).

Particulate matter mainly enters the human body via ingestion or inhalation. From the work within other fields dealing with particulate matter, such as ENMs and airborne particles, it can be anticipated that microplastics may exhibit different adverse effects in the human body, depending on the exposure route. This is related to a possible particle and chemical toxicity (Rist et al., 2018b - Paper II). When PS particles between 50 nm and 3 µm were fed to rats, translocation across the gastrointestinal tract and transport to the liver and spleen was observed in a size-dependent manner. Particles <300 nm were

found in the blood and those <100 nm reached the bone marrow (Jani et al., 1990). A low degree of uptake from the gastrointestinal tract was, however, also observed for 2 µm latex particles (Doyle-McCullough et al., 2007). Inhalation is the second major uptake route for particles and also relevant with regards to microplastics, as airborne microplastics are expected to constitute a source of exposure for humans (Prata, 2018). Airborne particulate matter with an aerodynamic diameter <2.5 µm ($PM_{2.5}$) is largely retained in the lungs and may translocate to the circulatory system (Feng et al., 2016). Accordingly, $PM_{2.5}$ has been associated with respiratory and cardiovascular diseases (Chen et al., 2016; Stone et al., 2007) and the central mechanism of adverse human health effects of $PM_{2.5}$ is most likely inflammation, along with oxidative stress and genotoxicity (Feng et al., 2016).

In comparison to particle-related toxicity, more is known about the toxicity of many plastic-associated chemicals (Rist et al., 2018b - Paper II). This includes residual monomers from plastic production as well as additives. Prominent examples are phthalates, which are commonly used plasticisers, and bisphenol A (BPA), which is a monomer of polycarbonate and also used as a plastic additive. Both phthalates and BPA have been found in urine, blood, breast milk and tissue samples, and they are suspected endocrine disrupting chemicals, which have been associated with reproductive and developmental abnormalities (Halden, 2010; Hauser, 2005; Swan, 2008). A general health concern is therefore understandable with regards to microplastics and associated chemicals. However, in order to evaluate the hazard connected to consuming microplastics from specific food products, it is essential to identify all important exposure pathways and compare them quantitatively.

There is still a high uncertainty related to the amount of microplastics that humans consume with their food. Only a limited number of studies have quantified microplastics in food products, and even for similar products results can differ substantially, as in the case of honey (Liebezeit and Liebezeit, 2013; Mühlschlegel et al., 2017). Maximum exposure levels per person annually, which have been estimated for different products, are 11,000 plastic particles from consuming shellfish (Van Cauwenberghe and Janssen, 2014), 1,000 from sea salt (Yang et al., 2015), 1,800 from beer and 5,800 from tap water (Kosuth et al., 2018). Recently, a special focus was put on microplastics in drinking water, following a report on widespread microplastic contamination of tap water in 14 countries worldwide (Kosuth et al., 2018). Plastic particles have since also been detected in groundwater wells and water treatment plants (Mintenig et al., 2019; Pivokonsky et al., 2018) as well as in bot-

tled water (Mason et al., 2018; Oßmann et al., 2018; Schymanski et al., 2018). The origin of microplastic contamination in water was mainly traced back to abrasion of plastics during different steps in the production process and the use of plastic bottles. This presents a change of perspective regarding the sources of human exposure to microplastics: moving from the aquatic environment as a rather diffuse source for microplastics, to much closer sources, which are directly related to plastic production and use. While it is questionable whether drinking water constitutes a significant exposure pathway for microplastics, these studies can contribute to broadening the perspective on human microplastic exposure.

So far, the debate has mainly focused on specific food products and thereby often failed to see the overall picture: plastics are such an integrated part of our lives that the main exposure is most likely related to the everyday use of plastic materials. It has therefore been postulated that the number of microplastics in food products and beverages is completely outweighed by the amount of plastic particles that humans are exposed to by the use of synthetic textiles, plastic packaging, food contact materials, kitchen appliances and building materials (Rist et al., 2018b - Paper II). Research into these sources is only in its beginning, but airborne microplastics, especially fibres, have been identified as an important exposure pathway in outdoor and indoor environments (Cai et al., 2017; Dris et al., 2017, 2016). Airborne particles are likely to be inhaled. Furthermore, it has been estimated that more than 68,000 airborne fibres, which settle on a plate during a regular meal, are ingested per person annually (Catarino et al., 2018). This clearly exceeds all maximum estimates for microplastics in individual food products. In order to approach a more complete picture of human exposure to microplastics, it is imperative to take all pathways into account and compare them quantitatively (Fig. 11). At the same time, it needs to be recognised that different sources and pathways are interlinked. This can, for instance, be illustrated for airborne fibres, which may be inhaled directly or ingested after settling on food products during use or during the production process. On a bigger scale, they can also contaminate the environment and end up in aquatic organisms used for human consumption (Fig. 11).

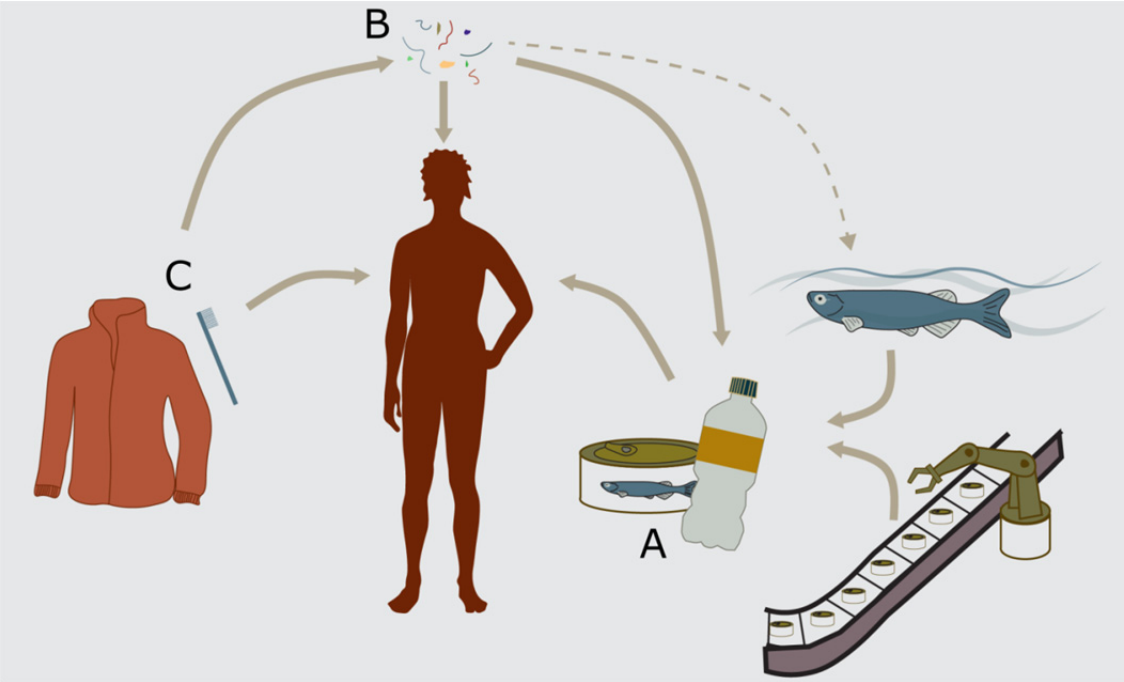

Figure 11. Humans are exposed to microplastics and associated chemicals through a variety of exposure pathways, including plastic particles in food products (A), airborne microplastics (B) and particles from a variety of everyday plastic materials (C). The major pathways need to be identified and their relative contribution to the overall exposure should be determined. Figure modified from Rist et al. (2018b - Paper II).

Also the exposure to plastic-associated chemicals is mainly related to the general usage of plastics and not to microplastics from food products. This has for instance been illustrated for BPA (Rist et al., 2018b - Paper II). Taking a high concentration of BPA that has been reported from microplastics in the field, and assuming that the maximum number of 11,000 particles that may be consumed with shellfish per person annually all carried BPA at this concentration, the theoretical annual exposure to BPA from consuming shellfish would be 0.034 ng (Rist et al., 2018b - Paper II). This is almost 40 million times less than the BPA concentration that humans have been estimated to take up by their general food consumption, mainly stemming from food contact materials (Gyllenhammar et al., 2012). Although this comparison is based on many assumptions, it indicates that consumption of microplastics from seafood most likely constitutes a minor source for plastic-associated chemicals to humans – a conclusion that has also been drawn by Smith et al. (2018) and the European Food Safety Authority (EFSA, 2016).

It seems like microplastics are detected wherever researchers look, in environmental samples as well as in products for human consumption. During

sampling and sample analysis, it is often very challenging to prevent contamination with plastic particles stemming from the air or the equipment that is used (Woodall et al., 2015). This increases the uncertainty with regards to the actual microplastic abundance in different matrices, but it also demonstrates the ubiquity of plastics around us. The root cause for this is the way in which plastics are currently produced, used and disposed of. Human exposure to microplastics from different sources and potential implications for human health are poorly understood and require more research. However, it can be expected that human microplastic exposure is closely connected to the general use of plastic materials. It is therefore important to keep the bigger perspective in mind and not only focus on microplastics in specific food products (Rist et al., 2018b - Paper II).

6.2 The notion of microplastics as a threat to humans and the environment

Pollution of global environments with microplastics has received enormous attention during the last decade, with a quickly increasing number of published studies within this field (Connors et al., 2017). Often, microplastics are perceived and depicted as a (potential) threat to organisms specifically and ecosystems in general, but there is no consensus on this within the scientific community. On the contrary, there are many active discussions and opinion articles supporting and challenging the notion of microplastics as a threat (Backhaus and Wagner, 2018; Burton, 2017; Galloway and Lewis, 2016; Hale, 2018).

Despite an increasing number of studies, knowledge on the effects of microplastics on organisms is still limited. Adverse effects as well as no effects on organisms have been reported, with few general trends (see Chapter 3). Often, the environmental realism of laboratory studies is criticised, questioning whether results can be extrapolated to the field (see Chapter 5.3). Within ecotoxicology there is the concept that the effect of a pollutant cascades from one level of biological organisation to the next (Fig. 5), meaning that effects that have been observed in individual organisms could eventually impact whole populations and ecosystems (Galloway et al., 2017). There are some indications of microplastic effects on higher levels, such as reduced fecundity (Balbi et al., 2017; Tallec et al., 2018) and behavioural changes, which might affect predator-prey interactions, bioturbation and nutrient cycling (Green et

al., 2016; Tosetto et al., 2016; Wright et al., 2013a). However, this is still poorly understood and data is too scarce to draw conclusions on the impact of microplastics on natural ecosystems.

In an attempt to evaluate the overall risk that microplastics pose on aquatic animals, Burns and Boxall (2018) made a species sensitivity distribution and concluded that based on currently available data, microplastics do not constitute a threat since the concentration at which 5% of the species in an ecosystem are harmed was found to be 3 orders of magnitude higher than the measured environmental concentration. In a similar analysis that focused on the marine environment and did not consider potential chemical toxicity, the predicted environmental concentration (PEC) of microplastics was also found to be below the predicted no effect concentration (PNEC) for both the pelagic and benthic ecosystem, indicating no general adverse effects of microplastics (Everaert et al., 2018). While it was predicted that it would remain like this for the pelagic ecosystem until the year 2100, the PEC may exceed the PNEC in the benthic ecosystem within the second half of the 21st century. It must be noted though that these models come with many uncertainties and only provide an indication of general trends. As discussed above, the PEC is likely underestimated with currently available methods. Also, the models give numbers for the aquatic or marine environment as a whole, but it is well established that microplastic abundance is highly heterogeneous. At pollution hotspots the PEC can already exceed the PNEC today (Everaert et al., 2018), making it questionable how meaningful such global analyses are.

Based on current knowledge, it is not possible to conclude to what extent microplastics constitute a threat to aquatic organisms and ecosystems, or humans, because there are too many knowledge gaps and uncertainties. Especially nanoplastics are a black box in this respect since virtually no information is available on their abundance in the environment. With regards to microplastic fate and effects, it can be expected that most uncertainties are epistemic, meaning that they can be reduced by more research in the future (Walker et al., 2003). However, it may also include inherent, or stochastic, uncertainties that are not reducible, similar to what has been identified for ENMs, due to spacial, temporal and species-specific variability (Grieger et al., 2009). This needs to be acknowledged but should not prevent active support in developing effective policies and measures to reduce plastic pollution. It is clear that once released to the environment, microplastics will persist for long periods of time and there are no feasible means to remove them. In the course of the last decade, a strong public opinion against plastic pollution has

formed, putting pressure on industries and governments (Dauvergne, 2018; Knoblauch et al., 2018; Syberg et al., 2018). This has also been supported by members of the scientific community (Rochman et al., 2015a, 2013a). The changing perception and opinion towards environmental plastic pollution in general and microplastics in particular might even be considered a new environmental norm (Dauvergne, 2018). This quickly drives societal actions, such as the recent bans of microbeads in rinse-off products in several countries worldwide, without waiting for a final scientific evaluation of hazards (Kramm et al., 2018).

6.3 Communicating a more holistic perspective on plastics

Plastic debris in the environment is a very heterogeneous type of contamination, comprising a variety of materials with many different properties. In order to structure a complex group, categories and definitions are made. With regards to plastics, this is mainly based on size, giving rise to 'microplastics' as a widely accepted term for plastic particles <5 mm (see Chapter 2.2). Other categories like nanoplastics and mesoplastics have not reached a similar consensus yet, but a continuous development towards clear definitions can be observed within this field (Gigault et al., 2018; Hartmann et al., 2018 - Paper VIII). Categories and definitions are arguably important to set the focus on a specific part of a problem, to enable comparisons between studies and in a regulatory context. However, we should also be aware that this can set somewhat arbitrary barriers (Hartmann et al., 2018 - Paper VIII) and it is easy to lose sight of the wider connections. For instance, when quantifying microplastics in environmental samples, the upper size limit usually is 5 mm, in accordance with the accepted definition. This does, however, not mean that plastic particles just outside this range, for example between 6 and 10 mm, constitute a different kind of pollution. As discussed in Chapter 2.2, size is the primary defining property for microplastics and for practical reasons it is unavoidable to choose a certain size range and set boundaries. Furthermore, size has been recognised as an important factor in the interaction of plastic particles with organisms (see Chapter 3). Still, it is important to keep in mind that microplastics are only one part of plastic pollution as a whole.

The disconnection between microplastics and plastics in general becomes apparent in the discussion on human health effects of microplastics in food and

beverages. This is largely focused on one aspect – the presence of microplastics in certain products – while ignoring the bigger picture. In this way, the general public may be highly concerned about findings of plastic particles in fish, but at the same time there is limited reflection on other, more direct sources, such as the plastic container that the fish is sold in (Rist et al., 2018b - Paper II). The difficulties of recognising this inconsistency is partly related to the fact that plastics are such an integrated part of everyday life that we do not reflect on our use of plastics. It is, however, also connected to a different risk perception of microplastics in food products and beverages on the one side and general plastic materials on the other. While the latter can be considered a voluntary and controlled risk, the former is more likely perceived as an imposed, uncontrolled risk and as such causes greater concern (Syberg et al., 2018).

Another factor to consider is exaggerated and sometimes misleading reporting of health risks from microplastics in the scientific literature and public media (Koelmans et al., 2017a; Rist et al., 2018b - Paper II). As researchers we need to be careful in the way we communicate potential risks of microplastics. While it is an important part of the scientific discussion to address possible broader implications of our findings, we should also take care not to make speculations that stretch too far from actual findings. Microplastic pollution is a sensitive topic, which quickly gets picked up by the general media and has the potential to influence decision making in politics and industries (Dauvergne, 2018). Already now legislative and societal actions are moving faster than scientific consensus on microplastics (Kramm et al., 2018). This can entail the risk of setting a focus that misses taking the most effective measures. Therefore, it is important that the scientific community supports decision making on the basis of current knowledge, while at the same time acknowledging knowledge gaps and working towards closing these.

7 Conclusions

Microplastic research is still a young and developing field and many knowledge gaps remain. This is especially the case for the comparatively new aspect of human exposure to microplastics and potential effects – a topic that to date leaves more open questions than answers. However, with regards to interactions between microplastics and aquatic organisms, and the interaction with chemicals, there is today a basic understanding that future research can build on.

Through the laboratory work and literature review in this thesis, it is documented that aquatic invertebrates of different life stages ingest a wide variety of plastic particles. The degree of ingestion as well as egestion can be influenced by many factors, including particle concentration, size, shape, biofouling, exposure time and food availability. Quantifying these processes is an important step in understanding the interactions between organisms and plastic particles, as well as explaining observed effects. Translocation of microplastics from the point of initial uptake to other tissues has been observed in few cases, but further investigation of underlying mechanisms on the basis of biological plausibility is needed. It can be expected that translocation strongly depends on the organism and particle properties, primarily particle size.

Microplastics have been found to cause effects on different levels of biological organisation, including cells, organs and tissues, whole organisms and possibly populations. Most laboratory studies, however, lack environmental realism in terms of particle concentrations and types, making it questionable whether results can be extrapolated to the field. For instance, most effects have been observed at concentrations significantly higher than those that are commonly found in the aquatic environment. At low, environmentally realistic concentrations animal physiology often is not affected, leading some studies to conclude that microplastics do not have a negative impact on aquatic ecosystems on a global scale. However, it is important to note that there are large site-specific differences and additional studies are needed that use more realistic exposure systems with regards to microplastic concentrations and particle types.

It is not trivial to conduct microplastic effect studies. Many influencing factors need to be considered to determine the cause of an effect. In this thesis, it is argued that it can help to have a high level of control over the exposure system. In parallel to more realistic exposure experiments, highly controlled

laboratory tests are therefore a useful tool for understanding basic mechanisms. They can also enhance the comparability between studies and the reproducibility of results, which is a major challenge to date. Similar to the work with engineered nanomaterials, microplastic exposure studies could greatly benefit from a detailed characterisation of the particles (regarding size, composition, density, charge and surface chemistry) and the exposure (aggregation/agglomeration, sedimentation and dispersion), as well as the implementation of natural particle controls and controls for chemical toxicity.

Plastics and chemicals are strongly interlinked due to chemicals within the plastic material as well as sorbed chemicals from the environment. This interaction with chemicals challenges the notion of microplastics as inert particles. Thus, plastic particles have a potential particle- and chemical-related toxicity. They can act as vectors for chemicals to organisms – a process that strongly depends on the properties of the particle, the chemical and environmental factors. In comparison to natural pathways of chemicals to organisms, such as natural inorganic particles, water and organic matter, plastics as vectors most likely play a minor role globally. This could, however, differ on a smaller scale, for instance at hotspots of plastic pollution.

While the majority of research on effects of microplastics focuses on aquatic organisms, potential human health impacts are receiving a higher degree of attention in public media. Especially the presence of microplastics in food products has evoked concerns about possible effects on human health and safe consumption of these products. However, studies on human health effects of consuming microplastics are virtually non-existent and human exposure to microplastics and associated chemicals is expected to be mainly related to the general use of plastic materials in everyday life. More research is needed that takes a broader perspective into account, quantifying all exposure pathways, and addressing the root of the problem: the way in which plastics are currently produced, used and disposed of. At the same time, immediate action to reduce present and prevent future plastic pollution of the environment is necessary. Therefore, the current societal and political focus on the problem is vital. In this context, it is essential that scientific findings are communicated in a balanced way: giving advice based on the state of knowledge and acknowledging knowledge gaps that need to be addressed by future research. In this way, policies can be supported, which direct the focus towards the most urgent issues and thus drive the implementation of effective measures.

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9 Papers

I **Rist, S.**, Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in *Daphnia magna* – Quantification of body burdens and assessment of feeding rates and reproduction. *Environmental Pollution*. 228, 398-407. doi:10.1016/j.envpol.2017.05.048

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VII Hartmann, N.B., **Rist, S.**, Bodin, J., Jensen, L.H., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as Vectors for Environmental Contaminants: Exploring Sorption, Desorption, and Transfer to Biota. *Integrated Environmental Assessment and Management*. 13, 488–493. doi:10.1002/ieam.1904

VIII Hartmann, N.B., Hüffer, T., Thompson, R., Hassellöv, M., Verschoor, A., Daugaard, A.E., **Rist, S.**, Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Heß, M., Ivleva, N.P., Lusher, A.L., Wagner, M., 2018. Are we speaking the same language? Towards a definition and categorization framework for environmental plastic debris. *Submitted.*

In this online version of the thesis, **paper I-VIII** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from.

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